BIO-TEK®

User's Guide

User's Guide to

KCjunior™

October 2002
Copyright © 2002
PN 5271000
Revision E
Bio-Tek Instruments, Inc.

Notices

BIO-TEK® INSTRUMENTS, INC.
Highland Park, Box 998
Winooski, Vermont
05404-0998 USA

Customer Service & Sales:

Phone:	(888) 451-5171 (toll free in the U.S.)
	(802) 655-4040 (outside the U.S.)
Fax:	(802) 655-7941
E-Mail:	sales@biotek.com
Internet:	http://www.biotek.com

Service/Technical Assistance Center (TAC):

Phone:	(800) 242-4685 (toll free in the U.S.)
	(802) 655-4740 (outside the U.S.)
Fax:	(802) 655-3399
E-Mail:	labtac@biotek.com

European Coordination Center

Bio-Tek® Instruments GmbH
Kocherwaldstr. 34
D-74177 Bad Friedrichshall
Germany

Phone:	+49 (0) 7136-9680
Fax:	+49 (0) 7136-968-111
E-Mail:	info@biotek.de

All Rights Reserved

Copyright © 2002, Bio-Tek® Instruments, Incorporated. This publication is protected by copyright and all rights are reserved. No part of this manual may be reproduced or transmitted in any form, or by any means electronic or mechanical, including photocopying and recording, for any purpose other than the purchaser's personal use without written permission of Bio-Tek® Instruments, Inc.

Printed in the United States of America.

Bio-Tek Part Number 5271000.

Trademarks

Bio-Tek® is a registered trademark, and KCjunior™, KCJr™, and OMNI™, are trademarks of Bio-Tek Instruments, Inc.

All other trademarks are the property of their respective holders.

Restrictions and Liabilities

Information in this document is subject to change, and does not represent a commitment by Bio-Tek Instruments, Inc. Changes made to the information in this document will be incorporated in new editions of the publication. No responsibility is assumed by Bio-Tek for the use or reliability of software or equipment that is not supplied by Bio-Tek, or its affiliated dealers.

Limited Software Warranty

Bio-Tek Instruments, Inc. ("Bio-Tek") warrants, for a period of ninety (90) days after the date of delivery, that all software manufactured and sold by Bio-Tek (the "Software") will conform, as to all substantial operational features, to Bio-Tek's then current published specifications (meaning those specifications in effect as of the date of purchase) and will be free of defects which, in Bio-Tek's sole judgment, substantially affect system performance. This Warranty is limited to the original purchaser (the "Purchaser") and cannot be assigned or transferred. All claims under this Limited Warranty must be made in writing or fax to Bio-Tek attention: Service Department.

The Purchaser must notify Bio-Tek in writing within ninety (90) days after the date of delivery of the Software to the Purchaser of any claim under this Limited Warranty. This 90 day period shall not be extended by the delivery or receipt of any subsequent modifications to the Software. Modifications to Software within a version ("Updates") are expressly excluded from the terms of this Warranty. If the Software is found to be defective by Bio-Tek, Bio-Tek's sole obligation under this Warranty is to use efforts consistent with Bio-Tek's regular business practices to attempt to remedy such defect. In no event shall Bio-Tek's liability under this warranty exceed the original purchase price of the Software.

This is a limited warranty. This warranty contains the entire obligation of Bio-Tek. No other warranties, express, implied, or statutory, are given. The implied warranties of merchantability and of fitness for any particular purpose are expressly excluded and do not apply to the software.

Under no circumstances shall Bio-Tek be liable to the purchaser or any other person for any direct, incidental, or consequential damages, whether arising out of breach of warranty or otherwise.

This warranty shall not be changed or modified in any way without the express written permission of an officer of Bio-Tek Instruments, Inc.

This Warranty shall be NULL AND VOID if any modifications are made to the Software by the Purchaser. Correction of difficulties or defects traceable to Purchaser's errors or system changes shall be billed to Purchaser at Bio-Tek's standard time and materials charge.

Licensing Agreement

LICENSE: **Single Use:** You have the non-exclusive right to use the enclosed programs. These programs can only be used by a single user. You may transfer the programs from one computer to another provided that the programs are used with only one computer at a time. You may not electronically transfer the programs from one computer to another. You may not distribute copies of the programs or documentation without the prior written consent of Bio-Tek Instruments, Inc., or as stipulated in a sub-licensing agreement.

Network Use: You have the non-exclusive right to use the enclosed programs. These programs can only be used on a single file server. The number of concurrent users on the network at any time may not exceed the number of licensed copies of this software. You may transfer the programs from one server to another provided that the programs are used with only one server at a time. You may not distribute copies of the programs or documentation without the prior written consent of Bio-Tek Instruments, Inc., or as stipulated in a sub-licensing agreement. You may not use, copy, modify, loan, lease or transfer the programs or documentation, or any copy except as expressly provided in this Agreement.

COPYRIGHT: The programs and the documentation are copyrighted. You may not copy any program or the documentation except as for loading the program into the computer/server as part of executing that program and for back-up purposes, respectively. You must reproduce and include the copyright notice on the back-up copy

TERMS: This license is effective until terminated. You may terminate it by destroying the programs and documentation and all copies thereof. This license will also terminate if you fail to comply with any term or condition of this Agreement. You agree upon such termination to destroy all copies of the programs and documentation.

Document Revision

Rev / Date	Changes
A / April, 1998	First Issue.
B / May, 1999	Updated *Defining General Information* to include precision and scientific notation. Updated *System Requirements* to include the Macintosh. Enhanced requirements for operating system and display resolution. Updated *Defining Protocols, Read Method Parameters* to include pathlength correction and spectral scanning. Updated *Defining Protocols, Reports* to include information on the new Curve Report. Updated *Reading Plates* to include collecting data from the EL301 reader. Added *Appendix A, Sample Reports.*

C / December, 1999	Added support throughout for fluorescence/ luminescence readers.
	Updated *Defining Protocols, Reports* to discuss enhanced Matrix Reports.
	Added new sample reports to *Appendix A.*
D / January, 2001	Updated *Installing the Software* to introduce a new method for password distribution and software registration.
	Updated *Setting Up the System* to introduce dual-reader support and the option to pre-configure Filter Wheel Cartridges for the FLx800.
	Updated *Defining Protocols* to introduce new features including: partial plate reading, well monitoring, continuous shaking for kinetic reads, reader calibration for spectral scan reads, filter wheel cartridge selection, the DILution function, and some new reporting options.
	Updated *Reading Plates* to discuss the new Results ID, using the reader's barcode scanner to retrieve the Plate ID, and selecting a filter wheel cartridge at run-time.
	Updated *Interpreting Results* to include selection of the Maximum Slope Unit of Measure.
	Added new sample reports to *Appendix A.*
E / October, 2002	Updated *Installing the Software* with the correct website address for software registration.
	Updated *Setting Up the System* to include filter position requirements for Dispense / Read protocols.
	Updated *Defining Protocols* to introduce new Read Mode selections, as well as the new Dispense / Read method type for the FLx800.
	Updated *Interpreting Results* to describe Dispense / Read results.
	Updated *Utilities* to describe the new Dispenser Control Panel feature for the FLx800.

Contents

Introduction 1-1

KCjunior 1-1
Technical Support 1-3
Visiting Bio-Tek's Website 1-4

Installing the Software 2-1

Read Me! 2-1
System Requirements 2-2
Removing an Earlier Version of KCjunior 2-3
To remove KCjunior: 2-3
Software Installation 2-5
To Install KCjunior: 2-5
To Start KCjunior: 2-12
Where to go from here 2-15
Password Distribution and Software Registration 2-16
Obtaining a password and registering the software online 2-17
Obtaining a password if you do not have Internet access 2-17
Registering the software if you do not have Internet access 2-17

Getting Started 3-1

KCjunior's Menu Structure 3-1
Toolbar 3-2
Results Menu 3-3
Protocol Menu 3-4
Utilities Menu 3-5
Setup Menu 3-6
Current Reader Menu 3-6
View Menu 3-7
Help Menu 3-7
Essential Information 3-9
Protocol Definition 3-9
Plate Data Acquisition 3-9
Data Reduction and Analysis 3-10
Quick Start 3-11
Guided Tour 3-12

Setting Up the System 4-1

Reader Support 4-1
Dual-Reader Support 4-1
Connecting the Reader to the Computer 4-2
Reader Setup 4-3
System Configuration 4-5
Well Identifiers 4-5
Reporting Options 4-7
General Default Information 4-8
Pathnames 4-9
Filter Wheel Cartridges 4-11

Defining Protocols 5-1

Overview 5-1
Working with Protocols 5-2
Where are Protocols Stored? 5-2
Opening an Existing Protocol 5-2
Creating a New Protocol 5-3
Modifying Protocols 5-4
Saving and Closing Protocols 5-4
Copying Protocols 5-5
Sharing Protocols and Databases 5-5
Importing Protocols 5-6
Defining Protocol Parameters 5-8
Defining General Information 5-8
Read Method Parameters 5-10
Templates 5-38
Curves 5-55
Cutoffs 5-58
Reports 5-61

Reading Plates 6-1

Overview 6-1
Working With Plates and Results 6-2
Where is Plate Data Stored? 6-2
Opening an Existing Results Record 6-2
Generating a New Set of Plate Data 6-3
Saving and Closing Results Records 6-4
Copying an Existing Results Record 6-4
Sharing Results Records and Databases 6-5
Importing Results 6-6

Reading a Plate 6-7
Reading a Plate Without a Protocol 6-7
Reading a Plate With a Protocol 6-10
Simulating a Plate Read 6-12
Reading Plate Data from a File 6-13
Collecting Data from an EL301 Microstrip Reader 6-14
Reader Runtime Errors 6-16
Comments/Warnings 6-16
Selecting a Filter Wheel Cartridge 6-17
Exporting Data 6-18
Viewing Results 6-24

Interpreting Results **7-1**

Overview 7-1
Well Notations 7-2
Raw Data 7-4
Raw Endpoint Read Data 7-4
Raw and Blanked Kinetic Read Data 7-5
Raw and Blanked Spectral Data 7-9
Raw MultiWavelength Read Data 7-13
Raw Dispense / Read Data 7-14
Blanked Data 7-16
Blanked Endpoint Read Data 7-16
Blanked MultiWavelength Data 7-17
Pathlength Correction 7-17
Transformation Results 7-19
Concentrations 7-20
The Standard Curve 7-21
Titer Curves 7-22
Interacting with the Graph 7-24
Printing and Copying the Graph 7-25
Editing the Standard Curve 7-25
Storing the Standard Curve 7-26
Labels 7-27
Template 7-28
Plate Formula Results 7-29
Statistics 7-30
Auto Sensitivity Results 7-32
Automatic Sensitivity Adjustment Process 7-33
Results Options 7-34
Plate Information 7-34
Well Information 7-36
Masking Wells 7-37
Modifying Simulation Data 7-38

Printing Reports 8-1

Reports ... 8-1
Print Preview ... 8-1
Print Results ... 8-2
Print Setup ... 8-3

Utilities 9-1

Overview ... 9-1
Incubation Control ... 9-2
Carrier Out/In ... 9-4
Dispenser Control Panel ... 9-5
Initializing the Dispenser ... 9-5
Priming the Fluid Lines ... 9-6
Purging the Fluid Lines ... 9-6
Setting the Injector Position ... 9-6
Syringe Setup ... 9-7
Diagnostics ... 9-8
Reader System Test ... 9-8
Universal Plate Test ... 9-10
Database Maintenance ... 9-14
A Note on Backup Procedures ... 9-14
Creating Archive Databases ... 9-15
Archiving Results Records and Protocols ... 9-15
Archiving Stored Curves ... 9-17
Archiving Test Results ... 9-18
Deleting Results Records and Protocols ... 9-19
Deleting Stored Curves ... 9-20
Deleting Stored Transformations ... 9-21
Deleting Test Results ... 9-22

Troubleshooting 10-1

Overview ... 10-1
Reader Errors ... 10-2
Communication Errors ... 10-2
Comments and Warnings ... 10-4

Appendix A, Sample Reports 11-1

Glossary 12-1

Index 13-1

Introduction

KCjunior

KCjunior interacts with and performs data analysis for select microplate readers. With KCjunior you can:

- Configure the software to communicate with two Bio-Tek readers, and switch easily between them.
- Retrieve optical density, fluorescence, or luminescence data from a wide variety of Bio-Tek's microplate readers.
- Perform Endpoint, Kinetic, MultiWavelength, and Spectral Scan reads, as well as Dispense/Read sequences. MultiWavelength, Spectral Scan, and Dispense/Read capabilities are reader-dependent.
- Perform Fluorescence and Luminescence reads with Bio-Tek's FLx800.
- Create protocols that contain instructions for the operation of the attached microplate reader, and for analyzing data retrieved from the reader.
- Display, print, export, save, and archive results.
- Export plate data to a file, or directly to a Microsoft Excel spreadsheet.
- Share protocol and results databases among multiple KCjunior users.
- Define two levels of formulas to manipulate raw or blanked data.
- Collect data from Bio-Tek's automated microplate readers in the standard 8x12 format, as well as in 2x3, 3x4, 4x6, 6x8, 6x10(T), 6x12(T), 8x12(T), 8x12(H), 8x12(M), and 16x24 formats (reader-dependent).
- Correct the wells on a microplate by a constant value to effectively eliminate background absorbance.

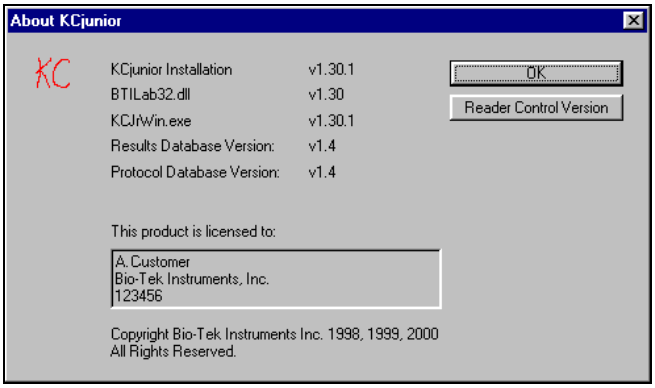
- Normalize absorbances to 1 cm by using the Pathlength Correction feature.
- Calculate the mean, standard deviation, and coefficient of variation of any group of wells on the plate.
- Generate a linear, quadratic, cubic, point-to-point, 2P, 4P, or cubic-spline standard curve.
- Calculate concentrations based on the curve generated using the present standards, or on a previously stored curve.
- Generate a curve using the sample dilutions as standards.
- Extend the abscissa range of a standard curve-fitting routine beyond the limits of the standard data points generated by the assay (“extrapolation”).
- Edit standard outliers and recalculate the curve.
- Run a Universal Plate Test on most readers and then view, print, and save the test results.
- Run a System Test on most readers and then view, print, and save the test results.
- Test communication between KCjunior and the reader before attempting to read a plate.
- Set default communication parameters appropriate for the attached reader.
- Control plate incubation (reader-dependent).

Technical Support

If KCjunior fails to function successfully, try to solve the problem(s) with the assistance of the **Reader Run-Time Errors, Comments and Warnings,** and **Troubleshooting** sections of the User's Guide or the Help system.

If the software still does not function successfully:

1. Select **Help|About KCjunior** from the **KCjunior** main screen. A dialog *resembling* the following appears:



2. Write down all of the version numbers and the serial number ("123456" in the example above).

3. Click the **Reader Control Version** button. A dialog *resembling* the following appears:

4. Write down the **BTI Autoreader Control** version.
5. Prepare a list of specific steps that can be used by Bio-Tek's Technical Assistance Center (TAC) to reproduce the problem.

6. Contact Bio-Tek Instruments. Be sure to provide a daytime phone or fax number, or email address where you can be reached.

Written Communication

Bio-Tek Instruments, Inc.
Technical Assistance Center
Highland Park, Box 998
Winooski, Vermont USA 05404-0998

Phone Support

Bio-Tek Instruments' Main Number:	(802) 655-4040
Technical Assistance Center, 8:30 am to 5:30 pm EST:	(800) 242-4685

Fax Support

Technical Assistance Center:	(802) 655-3399

Electronic Communication

Email:	labtac@biotek.com
Internet Site:	www.biotek.com

Visiting Bio-Tek's Website

www.biotek.com

If you have an Internet browser installed on your computer, you can visit Bio-Tek's World Wide Website, at **www.biotek.com.** Here you can find information on hardware and software products, read application notes, and more.

To visit the site from within KCjunior, select **Help|Bio-Tek on the Web,** then click on **Bio-Tek Home Page.** KCjunior automatically loads your browser and goes directly to the site.

Installing the Software

Read Me!

Complete the following to get KCjunior up and running:

1. Ensure that the computer on which KCjunior will be installed meets the **minimum system requirements.**
2. Remove the **previous version** of KCjunior from your computer, if one exists.
3. **Install** KCjunior.
4. Contact Bio-Tek to obtain a **password** to ensure continuous use of the software after the 30-day trial period.
5. **Register** your copy of KCjunior with Bio-Tek to record your warranty, and to receive information on upgrades and new products.
6. **Connect** your microplate reader(s) to the computer.
7. Configure the reader(s) and KCjunior so that they can **communicate** with each other.

Installing the Software covers steps 1 through 5.

See Chapter 4, *Setting Up the System* for information on connecting a reader to the computer, and configuring the reader and KCjunior.

System Requirements

For KCjunior to run successfully, you will need the following:

- Intel 486 processor / 33 MHz minimum (Pentium / 66 MHz or greater is recommended)
- Microsoft Windows 95, 98, 2000, NT, or XP operating system
- 8 MB of RAM minimum (16 MB or more is recommended)
- 20 MB of available hard drive space for the program files
- 1 MB for every set of 250 plates (96-well geometry) *stored on the hard drive*
- 3 1/2" high density diskette drive (1.44 MB) or CD ROM drive
- 16 color display / 640x480 (800x600 recommended)
- Keyboard, mouse, and serial ports

Important!

- KCjunior is capable of running any protocol on a computer meeting the minimum requirements if KCjunior is the only executing software.

  If additional software including user programs, browsers, network connections, scanning software and/or utilities is processing concurrently with KCjunior, overall performance may be affected.

Removing an Earlier Version of KCjunior

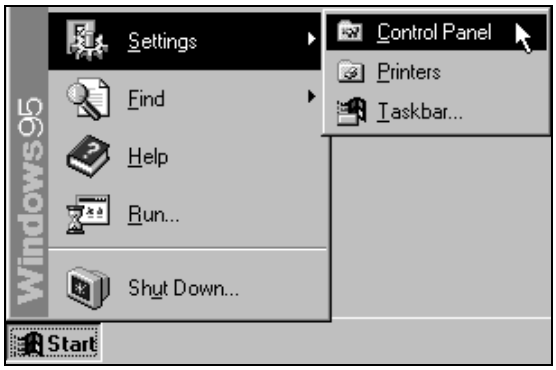
If you have an earlier version of KCjunior installed on your computer, you should remove it before installing the new version. For optimal performance, only one version of KCjunior should reside on your computer at any one time.

Note: This process does NOT remove existing protocol or results databases from your computer.

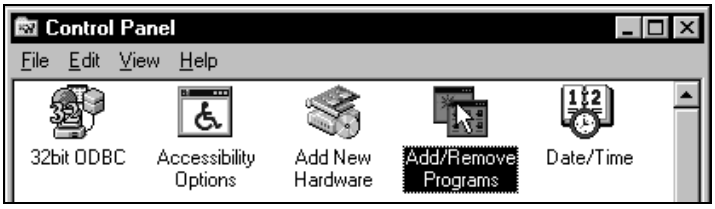
To remove KCjunior:

For optimal performance, only one version of KCjunior should reside on your computer at any one time.

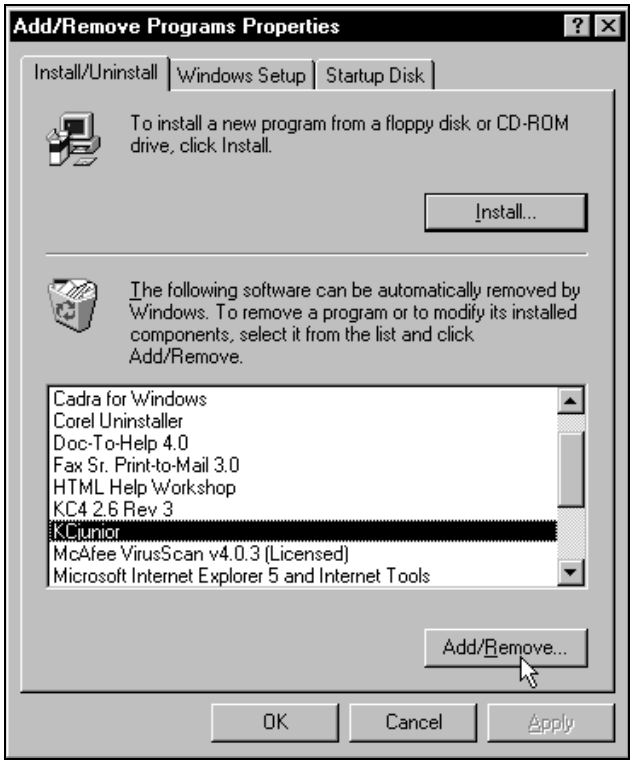
1. Start Windows.
   - **Note:** If you are prompted with the **Windows password** entry, enter a valid password. If you are **not** prompted with the Windows password entry, disregard this note.
2. Click the Windows **Start** button, then select **Settings, Control Panel.**



3. When the Control Panel folder opens, double-click on the **Add/Remove Programs** icon.



4. When the Add/Remove Programs Properties dialog opens, highlight KCjunior, then click **Add/Remove.**


Add/Remove Programs Properties
Install/Uninstall
Windows Setup
Startup Disk
To install a new program from a floppy disk or CD-ROM drive, click Install.
Install...
The following software can be automatically removed by Windows. To remove a program or to modify its installed components, select it from the list and click Add/Remove.
Cadra for Windows
Corel Uninstaller
Doc-To-Help 4.0
Fax Sr. Print-to-Mail 3.0
HTML Help Workshop
KC4 2.6 Rev 3
KCjunior
McAfee VirusScan v4.0.3 (Licensed)
Microsoft Internet Explorer 5 and Internet Tools
Add/Remove...
OK
Cancel
Apply

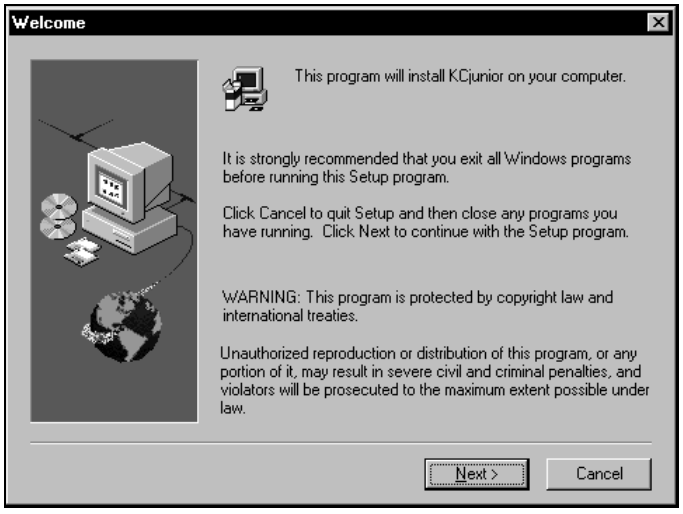
*The uninstall process leaves your **protocol** and **results databases** in their original location(s) on your computer.*

5. Follow the instructions on the screen to complete the uninstall process.

Software Installation

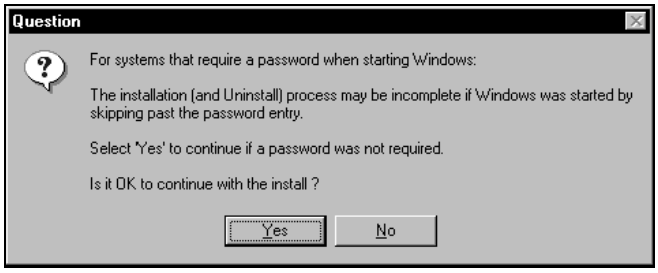
To Install KCjunior:

1. Start Windows.
   - **Note:** If you are prompted with the **Windows password** entry, enter a valid password. This helps to ensure a complete installation. If you are **not** prompted with the Windows password entry, disregard this note.
2. Close any unnecessary Windows programs.
3. Place **Disk 1** in a 3.5" diskette drive or the **CD** in the CD rom drive.
4. Click the Windows **Start** button and select **Run.**
5. When the Run dialog box appears, type `<drive>:\setup.exe`, where `<drive>` represents the letter corresponding to the diskette drive or CD rom drive.
   - Alternatively, use the **Browse** function to access the drive, then click on `setup.exe`.
6. Click **OK.** After a brief pause, the **Welcome** dialog appears:

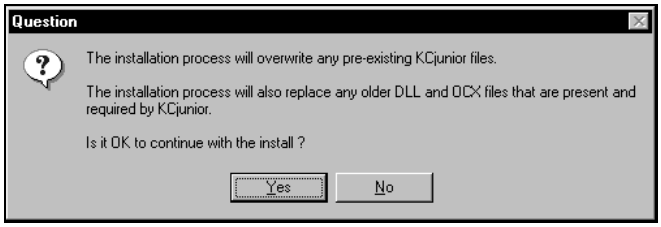

Welcome
This program will install KCjunior on your computer.
It is strongly recommended that you exit all Windows programs before running this Setup program.
Click Cancel to quit Setup and then close any programs you have running. Click Next to continue with the Setup program.
WARNING: This program is protected by copyright law and international treaties.
Unauthorized reproduction or distribution of this program, or any portion of it, may result in severe civil and criminal penalties, and violators will be prosecuted to the maximum extent possible under law.
Next >
Cancel

7. If you accept these terms, click **Next.** Otherwise click **Cancel** to discontinue the software installation.

- If you click **Next,** the following question appears:


Question
For systems that require a password when starting Windows:
The installation (and Uninstall) process may be incomplete if Windows was started by skipping past the password entry.
Select 'Yes' to continue if a password was not required.
Is it OK to continue with the install ?
Yes
No

8. If you were prompted for and entered a Windows password, **or** if you were not prompted for this password, click **Yes.**
   - If you were prompted for a Windows password but did not enter one, click **No** and go back to Step 1.
   - If you click **Yes,** the following warning may appear:


Question
The installation process will overwrite any pre-existing KCjunior files.
The installation process will also replace any older DLL and OCX files that are present and required by KCjunior.
Is it OK to continue with the install ?
Yes
No

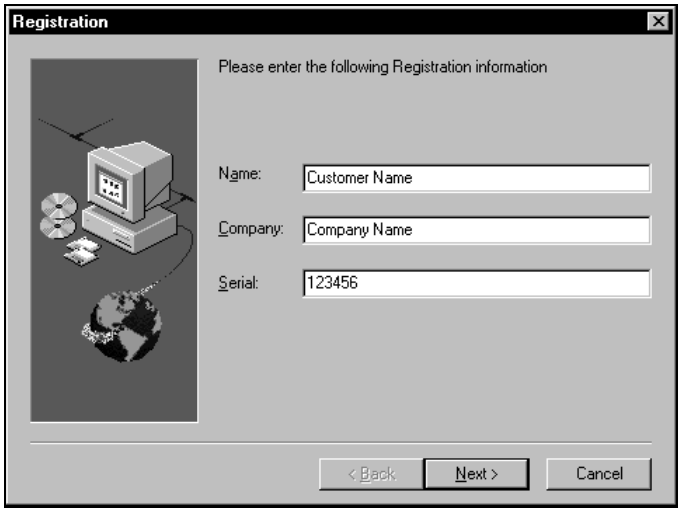
9. Click **Yes** to continue with the installation, or **No** to cancel.
   - If you click **Yes,** the **Software License Agreement** appears:

Software License Agreement
Please read the following license agreement. Use the scroll bar to view the rest of this agreement
BIO-TEK® INSTRUMENTS, INC.
LICENSING AGREEMENT
YOU MUST CAREFULLY READ ALL THE TERMS AND CONDITIONS OF THIS AGREEMENT PRIOR TO INSTALLATION. INSTALLING THIS SOFTWARE INDICATES YOUR ACCEPTANCE OF THESE TERMS AND CONDITIONS. If you do not agree with these terms and conditions, return the unopened package to Bio-Tek Instruments, Inc.
LICENSE: Single Use: You have the non-exclusive right to use the enclosed programs. These programs can only be used by a single user. You may transfer the programs from one computer to another provided that the programs are used with only one computer at a time. You may not electronically transfer the programs from one computer to another. You may not distribute copies of the programs or documentation without the prior written
Do you accept all the terms of the preceding license agreement? If so, click on the Yes push button. If you select No, Setup will close.
< Back
Yes
No

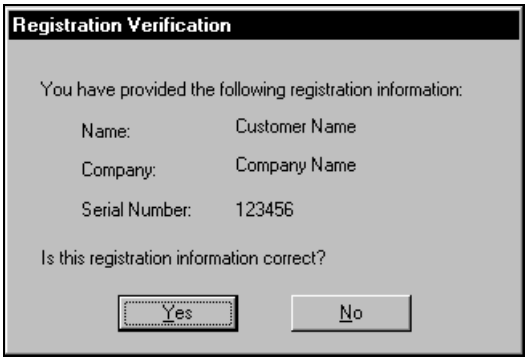
10. Click **Yes** if you accept the terms. Otherwise, click **No** to discontinue the installation.

*If you are running KCjunior in **trial mode** and do not have a serial number, enter any number in the Serial Number field.*

- If you click **Yes,** the **Registration** dialog appears:



11. Enter your name, company name, and the KCjunior serial number, then click **Next.**
    - The Registration Verification dialog appears:



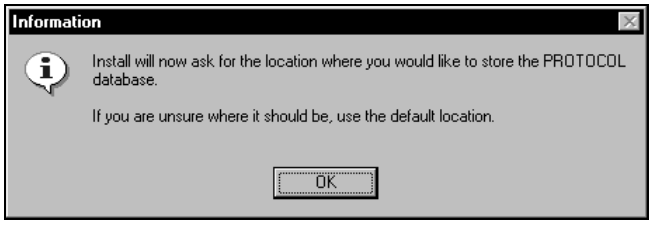
12. Click **Yes** to confirm and continue, or click **No** to make any changes.

- The **Choose Destination Location** dialog appears:

Choose Destination Location
Setup will install KCjunior in the following directory.
To install in this directory, click Next.
To install in a different directory, click Browse and select another directory.
Click Cancel to exit the Setup program.
Destination Directory
C:\KCjunior
Browse...
< Back
Next >
Cancel

13. The default location for the software is **C:\KCjunior.** Click **Next** to accept the default location, or click **Browse** to specify a different location.

- If you click **Next,** an informational dialog appears:


Information
Install will now ask for the location where you would like to store the PROTOCOL database.
If you are unsure where it should be, use the default location.
OK

14. Click **OK** to acknowledge the information.

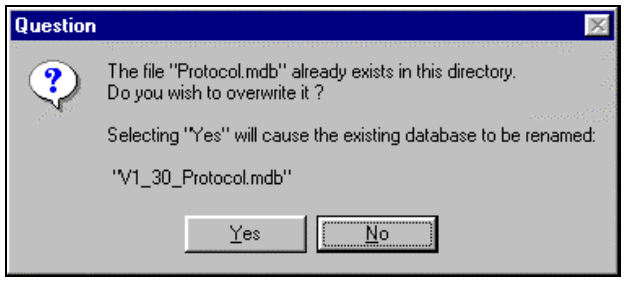
*A **protocol** contains instructions for controlling an attached microplate reader and information for analyzing plate data.*

*Protocols are stored in the **Protocol Database.** This database is typically stored on a computer's hard drive, or on a network to which the computer is attached.*

- The **Choose Protocol Database Location** dialog appears:

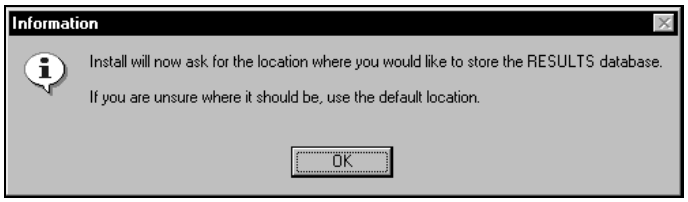
Choose Protocol Database Location
Select a directory where you would like the Protocol database to reside.
To install in this directory, click Next.
To install in a different directory, click Browse and select another directory.
Click Cancel to exit the Setup program.
Destination Directory
C:\KCjunior\Database
Browse...
< Back
Next >
Cancel

15. The default **Protocol Database Location** is the **\Database** directory within the KCjunior directory that you specified in step 13.
    - Click **Next** to accept the default location, or click **Browse** to specify a different location. **Note:** This location can be changed later from within KCjunior.
    - If a protocol database named PROTOCOL.MDB already exists in this location, the database is **renamed** and an empty PROTOCOL.MDB is installed.


Question
The file "Protocol.mdb" already exists in this directory.
Do you wish to overwrite it ?
Selecting "Yes" will cause the existing database to be renamed:
"V1_30_Protocol.mdb"
Yes
No

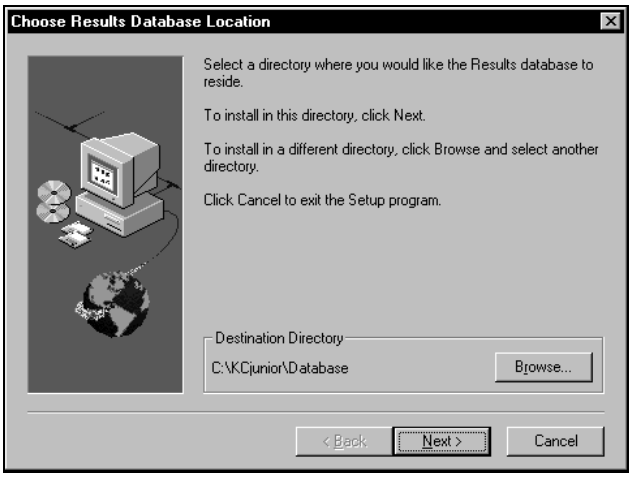
➢ Click **Yes** to rename the existing protocol database and continue. Click **No** to return to the **Choose Protocol Database Location** dialog.

- After the protocol database location has been selected, another informational dialog appears:

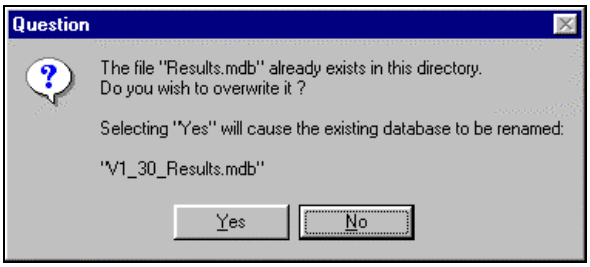

Information
Install will now ask for the location where you would like to store the RESULTS database.
If you are unsure where it should be, use the default location.
OK

16. Click **OK** to acknowledge the information.
    - The **Choose Results Database Location** dialog appears:

*Data for each plate is stored in a Results Record in the **Results Database.** This database is typically stored on a computer's hard drive, or on a network to which the computer is attached.*

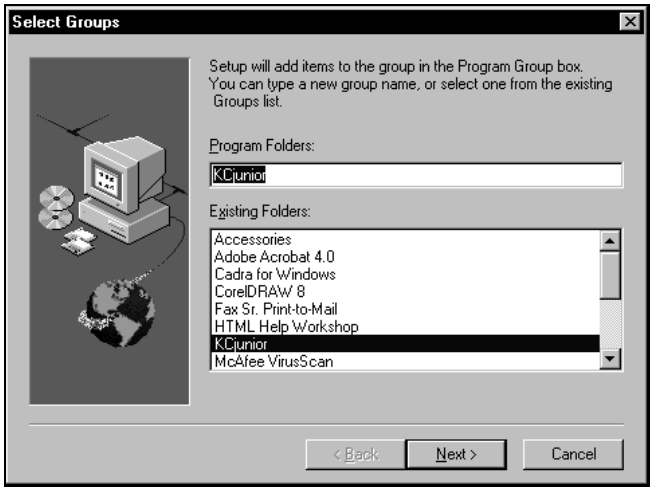

Choose Results Database Location
Select a directory where you would like the Results database to reside.
To install in this directory, click Next.
To install in a different directory, click Browse and select another directory.
Click Cancel to exit the Setup program.
Destination Directory
C:\KCjunior\Database
Browse...
< Back
Next >
Cancel

17. The default **Results Database Location** is the **\Database** directory within the KCjunior directory that you specified in step 13.
    - Click **Next** to accept the default location, or click **Browse** to specify a different location. **Note:** This location can be changed later from within KCjunior.
    - If a results database named RESULTS.MDB already exists in this location, the database is **renamed** and an empty RESULTS.MDB is installed.


Question
The file "Results.mdb" already exists in this directory.
Do you wish to overwrite it ?
Selecting "Yes" will cause the existing database to be renamed:
"V1_30_Results.mdb"
Yes
No

- After the results database location is selected, the software installation begins.

18. Toward the end of the installation, the **Select Groups** dialog appears:



19. Click **Next** to accept the default program folder name, or type in a new one.
20. When **Setup** is complete, KCjunior instructs you to restart your computer.
    - KCjunior will automatically restart your computer (recommended), or you can choose to wait and restart it yourself.

      Choose an option, then click **Finish.** KCjunior is now installed on your computer.

To Start KCjunior:

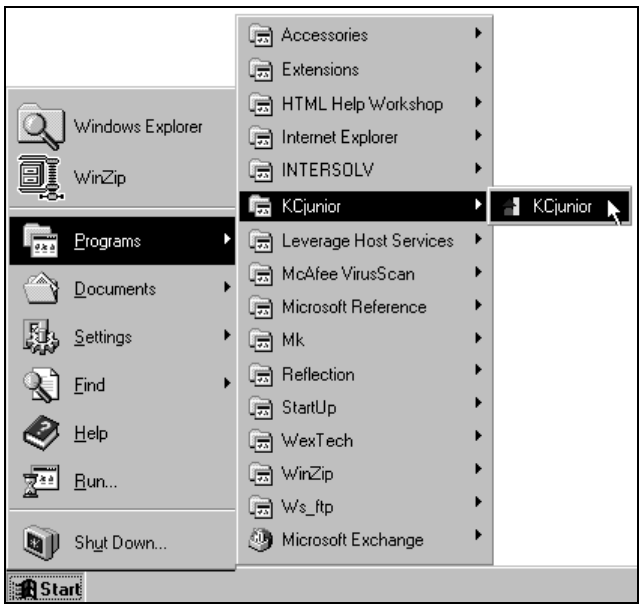
1. From the Windows **Start** menu, access the **Programs** menu, then the **KCjunior** menu. Click on KCjunior.

To create a KCjunior shortcut icon for your Desktop:

➢ *Open Windows Explorer. Make the window small so you can see the Desktop in the background.*

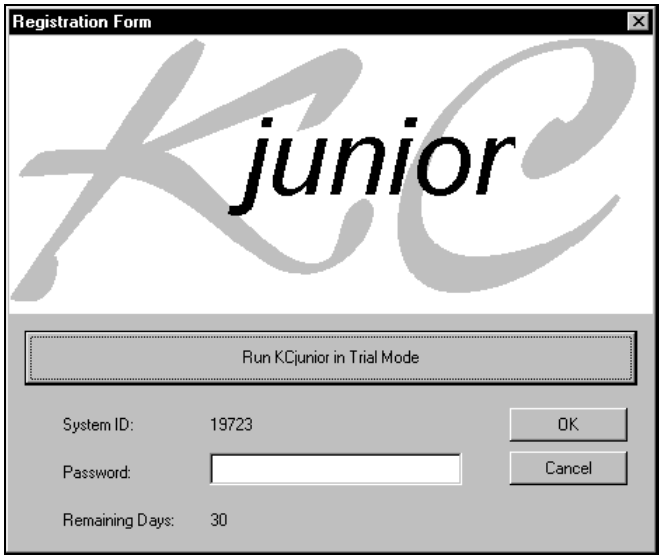
➢ *In the KCjunior folder, click and hold the **right** mouse button on KCJrWin.exe. Drag the item onto the Desktop and release the mouse button.*

➢ *Select **Create Shortcut(s) Here**.*



2. If you have not yet entered your KCjunior password, the **Registration Form** dialog appears:

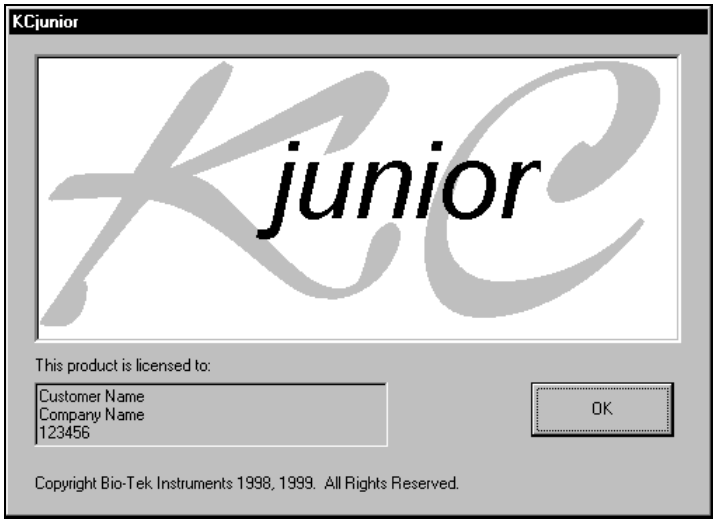
*You have **30 days** from the date of installation to use KCjunior without a password*



You have 30 days from the date of installation to use KCjunior without a password. See **Password Distribution and Software Registration** on page 2-16 for more information.

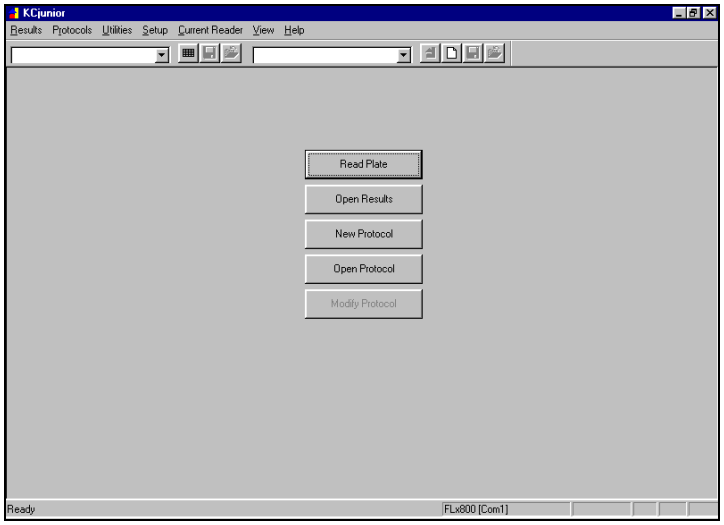
3. Click **Run KCjunior in Trial Mode** if you have not yet obtained a password.

   The KCjunior product information dialog appears:



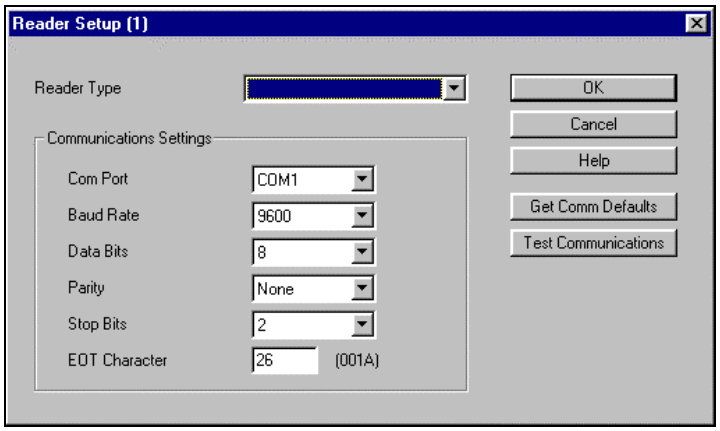
4. Click **OK** to continue.

   An initialization message appears, then after a few moments the KCjunior main screen appears:



5. The **Reader Setup** dialog automatically appears the first time KCjunior is started following software installation:

The reader does not need to be connected to the computer to specify a Reader Type.



If this dialog appears, select a **Reader Type.**

- The **Reader Type** represents the name of the reader to be controlled by KCjunior.
- Click ▼ to drop down the list, then click on the desired reader.

  Important notes:

  - Select the appropriate Reader Type **before defining protocols,** even if the reader is not actually connected to the computer.
  - **Choose carefully** -- it is important to select the correct model. For example, be sure to select "ELx808I, EL808I" for an 808 with the incubation option.
  - Select "PowerwaveX" for any **PowerWaveX** model other than the PowerWave XS. Do *not* select "PowerWave340" or "PowerWave340I" for a PowerWaveX 340 instrument.
  - KCjunior can be configured to communicate with any **two** Bio-Tek readers, if the computer has two serial ports. See "Reader Support" in *Setting Up the System* for more information.

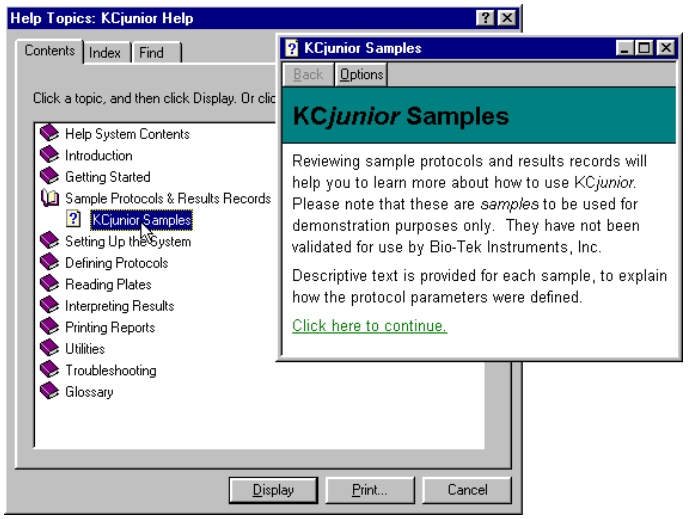
Select the reader type "PowerWaveX" for any PowerWaveX reader model other than the PowerWave XS.

- Click **OK** to continue.

  If necessary, KCjunior will provide instructions for setting additional communication parameters. See "Reader Setup" in *Setting Up the System* for more information.

Where to go from here

- See **Password Distribution and Software Registration** on page 2-16 to find out how to ensure continuous use of KCjunior after the 30-day trial period and how to register your software with Bio-Tek Instruments.
- Review the next section, **Getting Started,** to:
  - Learn about KCjunior's menu structure, and how to navigate around the **Help** system.
  - Review **essential information** – what you should know before using KCjunior.
  - Get started with the **Quick Start.**
  - Take a **guided tour** to learn how to create protocols, read plates, interpret results, and more.
- Examine **sample protocols** and **results records,** and read the corresponding **assay descriptions:**
  - From the KCjunior main screen, select **Help|Help Topics.**
  - Double-click on **Sample Protocols and Results Records,** then double-click again on **KCjunior Samples.**
  - The **KCjunior Samples** help will appear; follow the directions provided.

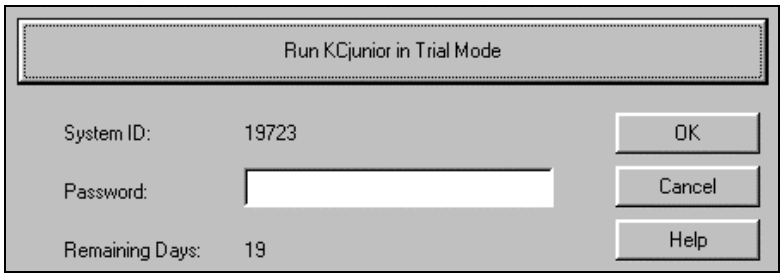

Help Topics: KCjunior Help
Contents
Index
Find
Click a topic, and then click Display. Or clic
Help System Contents
Introduction
Getting Started
Sample Protocols & Results Records
KCjunior Samples
Setting Up the System
Defining Protocols
Reading Plates
Interpreting Results
Printing Reports
Utilities
Troubleshooting
Glossary
Display
Print...
Cancel
KCjunior Samples
Back
Options
KCjunior Samples
Reviewing sample protocols and results records will help you to learn more about how to use KCjunior. Please note that these are samples to be used for demonstration purposes only. They have not been validated for use by Bio-Tek Instruments, Inc.
Descriptive text is provided for each sample, to explain how the protocol parameters were defined.
Click here to continue.

Password Distribution and Software Registration

You have 30 days from the date of installation to use KCjunior without a **password.** After 30 days, KCjunior will require you to enter a unique password in order to use the software. This password can only be obtained from Bio-Tek Instruments, Inc.

If you are connected to the Internet, you can get your password online and **register** your copy of KCjunior at the same time. Registering your software with Bio-Tek records your warranty and ensures that you will receive information on upgrades and new products.

Before you contact Bio-Tek, record the System ID from KCjunior's opening screen.



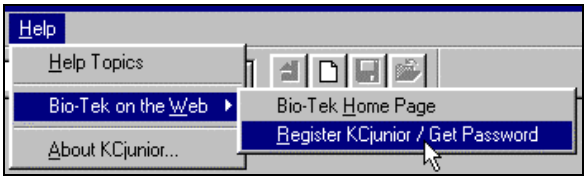
The **System ID** is an identifier that is unique to your computer. Bio-Tek will create a password for you based on this number.

When you receive your password, enter it in the **Password** field shown above then click **OK**.

- This password only needs to be entered once. KCjunior will not ask you for it again.
- If you need to **reinstall** KCjunior on the same computer, this password will still be valid.
- This password will only work with your computer. Contact Bio-Tek with any questions.

Obtaining a password and registering the software online

If KCjunior and your Internet browser are on the same computer, launch KCjunior and select **Help|Bio-Tek on the Web, Register KCjunior / Get Password.**



KCjunior will launch your browser and go directly to Bio-Tek's website. A **Password Distribution / Software Registration** form will appear. Follow the directions to fill out and submit the form.

If your computer is not connected to the Internet, enter this address into your browser: http://www.biotek.com/labcore.htm. Click the Software Registration link to access the form.

Obtaining a password if you do not have Internet access

Call, fax, or email Bio-Tek's Customer Service department. Tell them you wish to obtain a password for KCjunior, and give them your System ID.

(888) 451-5171

To call from anywhere within the United States, except from Vermont.

(802) 655-4040

To call from outside the United States, or from within Vermont.

(802)655-7941

To fax from anywhere.

sales@biotek.com

Email address.

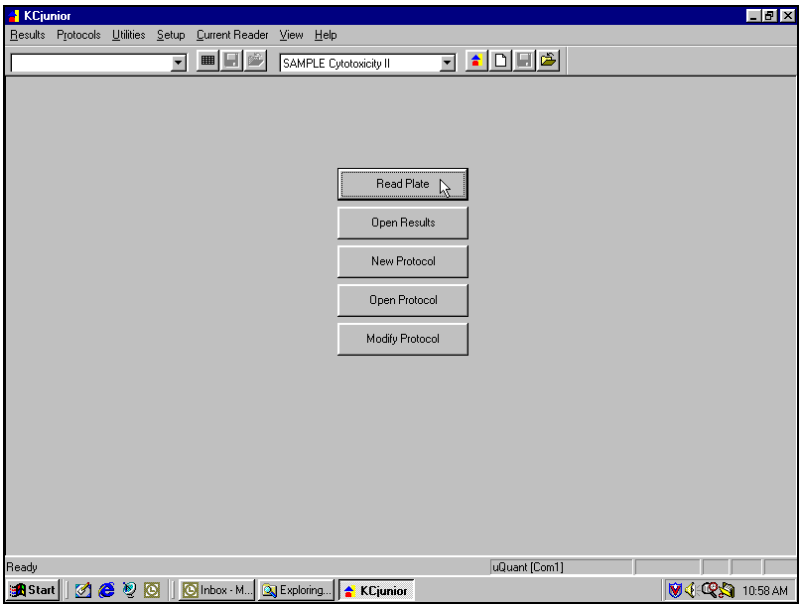
Registering the software if you do not have Internet access

Be sure to register your copy of KCjunior with Bio-Tek to record your warranty, and to receive information on upgrades and new products. If you cannot register online, you can do so by filling out and mailing the postage-paid registration card attached to the Customer Assurance Plan included in your software package.

Getting Started

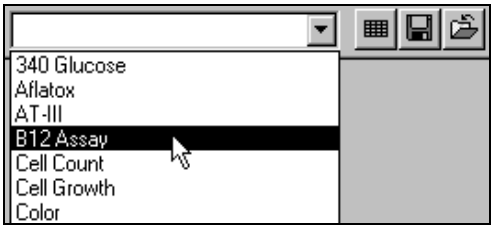
KCjunior's Menu Structure

KCjunior's functions are controlled through the **main screen,** shown below:



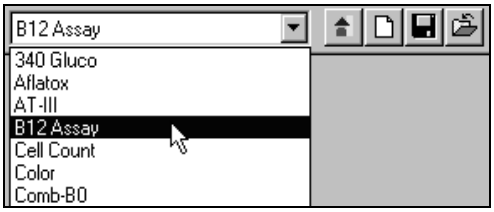
Toolbar

Use the icons on the left side of KCjunior's toolbar when working with **results records:**



- A **results record** contains the raw data retrieved from the microplate reader and related information such as Results ID, Plate ID, plate read time, status, and temperature.
- Click to open a list of existing results records.
- Click to read a new plate.
- Click to save the current results record.
- Click to close the current results record.

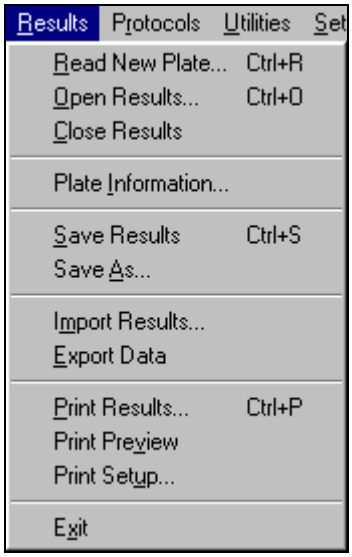
Use the icons on the right side of the toolbar when working with **protocols:**



- A **protocol** contains instructions for operating the attached reader and for performing data reduction on the raw data retrieved from the reader.
- Click to open a list of existing protocols.
- Click to modify the open protocol.
- Click to create a new protocol.
- Click to save the current protocol.
- Click to close the current protocol.

Results Menu

The **Results Menu** contains functions for acquiring and processing microplate data.



Read New Plate	Read a new plate.
Open Results	Open an existing results record.
Close Results	Close the open results record. If the record has not yet been saved, KCjunior provides the option to save before closing.
Plate Information	Open a dialog containing information about the plate, such as ID, description, status, and creation date and time.
Save Results	Save the results record.
Save As	Save the open results record under a new Results ID.
Import Results	Import results records from a stored Results Database into the one currently in use.
Export Data	Export plate data to a text file or directly into a Microsoft Excel spreadsheet.
Print Results	Print results according to reporting options specified in the Protocol.
Print Preview	See what the reports look like on the screen before printing.

Print Setup	Change the printer, paper size or source, or page orientation.
Exit	Close KCjunior. If a results record is open and unsaved, KCjunior provides the option to save before exiting.

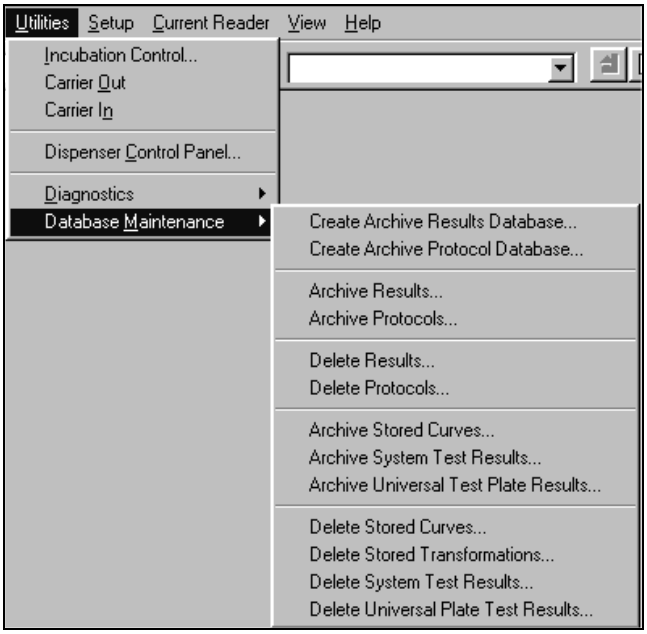
Protocol Menu

The **Protocol Menu** contains functions for creating, modifying, saving, and importing assay protocols.

New Protocol	Create a new protocol.
Open Protocol	Open an existing protocol.
Close Protocol	Close the open protocol. If the protocol has not yet been saved, KCjunior provides the option to save before closing.
Modify Protocol	Modify the current open protocol.
Save Protocol	Save the open protocol.
Save As	Copy the current open protocol to a new protocol with a different name.
Import Protocols	Import protocols from a stored Protocol Database into the one currently in use.

Utilities Menu

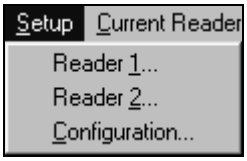
The **Utilities Menu** contains special tasks, including reader diagnostics and database maintenance.



Incubation Control	If the attached reader supports incubation, turn the incubator on/off, and set an incubation duration.
Carrier Out/In	Open / close door or extend / retract carrier.
Dispenser Control Panel	Perform setup, prime, and purge tasks specific to the FLx800 with the fluid injection capability.
Diagnostics	Perform system and alignment/accuracy tests on the attached reader (reader-dependent).
Database Maintenance	Create Results and Protocol Databases for archiving. Archive or delete results, protocols, stored curves and reader test results.

Setup Menu

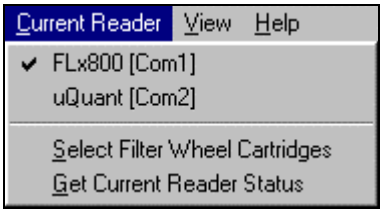
The **Setup Menu** provides access to the reader setup and system configuration features.



Reader 1 or 2	Define communication settings for the reader(s) attached to the computer.
Configuration	Define various default configuration parameters, including Well IDs, Reporting Options, Pathnames, and Filter Wheel Cartridges.

Current Reader Menu

If two readers are connected to the computer, use the **Current Reader Menu** to switch between them before working with protocols or reading plates.



(Reader 1)	Designate the first reader as the Current Reader.
(Reader 2)	Designate the second reader as the Current Reader.
Select Filter Wheel Cartridges	This option is only available if a fluorescence/ luminescence reader (like the FLx800) is defined as Reader 1 or Reader 2. Use it to choose from a predefined set of filter wheel cartridges.
Get Current Reader Status	Retrieve the current status of the attached reader. For example, if the reader encounters an error, select Get Current Reader Status to retrieve the error code.

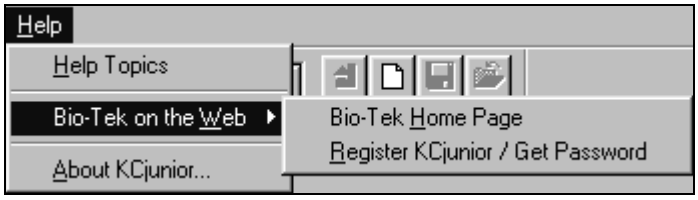
View Menu

The **View Menu** provides options for controlling the amount of information displayed within the main screen.

Toolbar	Show or hide the toolbar at the top of the screen. The default is *show*.
Status Bar	Show or hide the status bar at the bottom of the screen. The default is *show*.

Help Menu

The Help Menu provides access to KCjunior's help system and to Bio-Tek's internet site.

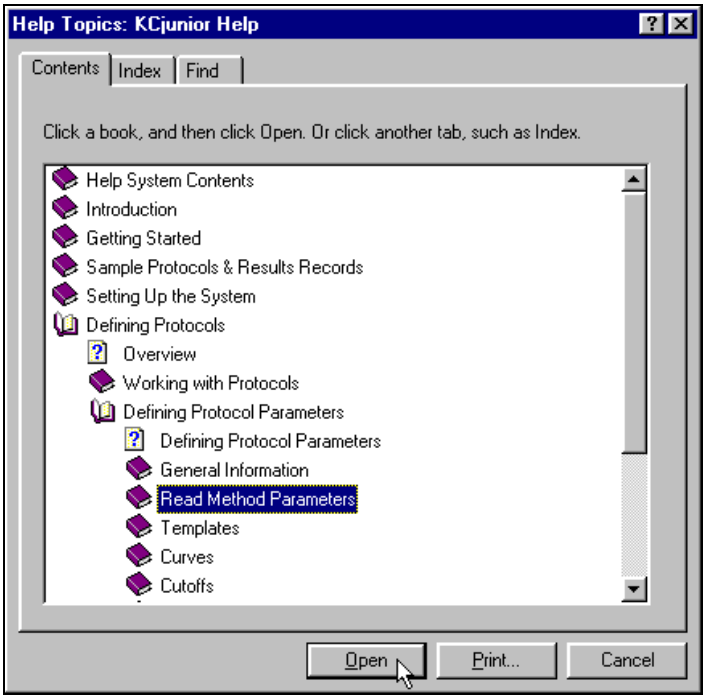


Help Topics	Access KCjunior's help system (Contents, Index, and Find).
Bio-Tek on the Web	Go to Bio-Tek's home page or register the software and get your password online.
About KCjunior	Retrieve software version information. This is useful if contacting Bio-Tek for support.

Navigating the Help System

Most KCjunior dialogs contain a **Help** button that, when clicked, opens an informational Help dialog.

In addition to this context-sensitive help, KCjunior provides access to the entire Help system. From the main screen, select **Help|Help Topics:**



- Double-click on a book to see a topic's sub-topics.
- Double-click on a page to view the text for a topic.
- Highlight a topic then click **Open** to view the text for the topic.
- Click the **Index** tab to perform a keyword search.
- Click the **Find** tab to search the entire help system for a specific word or phrase.
- Click **Print** to print the text for a topic.
- Click **Cancel** to close the Help Topics dialog and return to KCjunior.

Essential Information

KCjunior allows you to define instructions for acquiring microplate data and for reducing and analyzing that data.

Protocol Definition

The minimum required instructions for plate data acquisition are the **read method parameters.** These parameters can be combined with more detailed data reduction instructions within a **protocol.** In addition to storing read method parameters, a **protocol** stores templates, curve-fitting parameters, cutoff criteria, and reporting options.

While a fully defined protocol is useful for controlling a microplate reader and for performing data analysis, it is not a *requirement* that a protocol be defined prior to plate reading. KCjunior can perform **fast reads** using just the basic, user-defined read method parameters.

Plate Data Acquisition

KCjunior provides three methods for acquiring plate data: retrieve results from a microplate reader, read data in from an existing file, or manually enter simulation data.

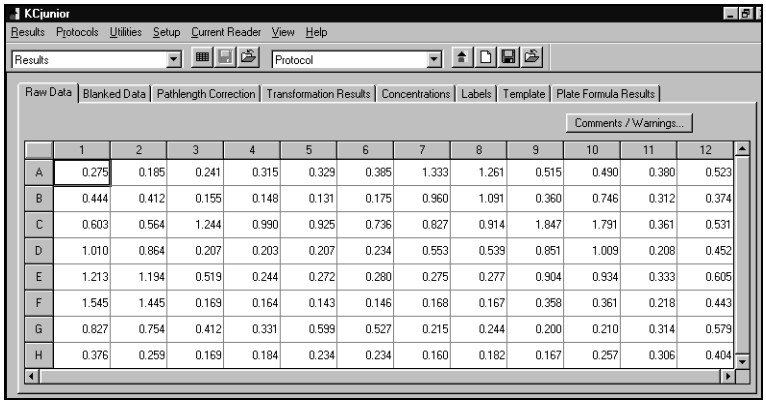
Refer to the following sections under **Reading a Plate** starting on page 6-7 for more information:

- "Reading a Plate Without a Protocol"
- "Reading a Plate With a Protocol"
- "Reading Plate Data from a File"
- "Simulating a Plate Read"
- "Collecting Data from an EL301 Microstrip Reader"

Data Reduction and Analysis

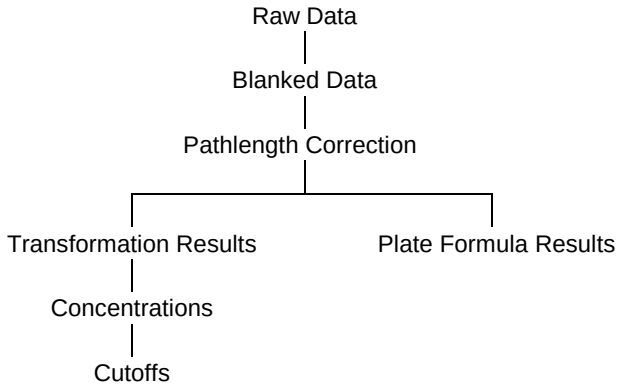
After data reduction is performed on microplate data, results are displayed in the **Raw Data** format.

Depending on the protocol definition, **Blanked Data, Pathlength Correction, Transformation Results, Concentrations, Cutoffs, Template,** and/or **Plate Formula Results** may also be displayed:



	1	2	3	4	5	6	7	8	9	10	11	12
A	0.275	0.185	0.241	0.315	0.329	0.385	1.333	1.261	0.515	0.490	0.380	0.523
B	0.444	0.412	0.155	0.148	0.131	0.175	0.960	1.091	0.360	0.746	0.312	0.374
C	0.603	0.564	1.244	0.990	0.925	0.736	0.827	0.914	1.847	1.791	0.361	0.531
D	1.010	0.864	0.207	0.203	0.207	0.234	0.553	0.539	0.851	1.009	0.208	0.452
E	1.213	1.194	0.519	0.244	0.272	0.280	0.275	0.277	0.904	0.934	0.333	0.605
F	1.545	1.445	0.169	0.164	0.143	0.146	0.168	0.167	0.358	0.361	0.218	0.443
G	0.827	0.754	0.412	0.331	0.599	0.527	0.215	0.244	0.200	0.210	0.314	0.579
H	0.376	0.259	0.169	0.184	0.234	0.234	0.160	0.182	0.167	0.257	0.306	0.404

Results calculation is structured as follows:



- The basis for calculations begins with the **Raw Data.**
- If blank subtraction is performed on the Raw Data, **Blanked Data** becomes the basis for calculating Transformation Results, Plate Formula Results, Concentrations, and Cutoffs.
- If **Pathlength Correction** is enabled to normalize absorbances to 1 cm (MultiWavelength reads only), the corrected absorbances become the basis for calculating Transformation Results, Plate Formula Results, Concentrations, and Cutoffs.

- If Transformation Formulas are applied, **Transformation Results** become the basis for calculating Concentrations and Cutoffs.
- If **Concentrations** are calculated, they become the basis for Cutoffs evaluation.
- **Notes:**
  - For Kinetic protocols, the **Maximum Slope** is the basis for calculating transformations, for curve calculation (if there are no transformations), and for cutoff evaluation (if there are no transformations or concentrations).
  - For **Dispense/Read** protocols (FLx800), Raw Data is the only result. KCjunior does not support blank well subtraction, pathlength correction, transformations, curve-fitting, or cutoffs for this read method type.

Quick Start

When using KCjunior, you will typically follow some version of the steps shown below:

1. Start KCjunior.
2. Connect a microplate reader to the computer.
3. Select **Setup|Reader** to define reader setup parameters.
4. Select **Setup|Configuration** to set default system parameters.
5. Select **Protocol|New Protocol** to create a new protocol, or **Protocol|Open Protocol** to open an existing one.
   - Define **read method** parameters, including read method type, wavelengths, and plate geometry.
   - Create a **Well ID** template.
   - Define **Curve** parameters and **Cutoff** criteria (if required).
   - Define **Reporting** options.
6. Select **Plate|Read Plate** to read a microplate.
7. Review results.
8. Select **Plate|Print Results** to print reports.
9. Select **Plate|Save Results** to save the information.
10. Select **Utilities|Database Maintenance** to archive protocol and plate data.

Guided Tour

Welcome to the *Guided Tour to KC*junior. This tour takes approximately 20 minutes, and shows you how to do the following:

- Prepare the **microplate reader.**
- Define and test **communication** parameters.
- Create a simple quantitative **protocol.**
- Design Well ID, Dilution, Transformation, and Sample ID **templates.**
- Define **curve-fitting** parameters.
- Define **cutoff** criteria.
- Select **reports.**
- **Read** a plate.
- Interpret **results.**
- Print **reports** and store **results.**

Let KCjunior's Help system guide you on the tour.

Refer to the User's Guide when taking the tour, or let the KCjunior Help system guide you. To access the guided tour in the help system:

1. Start KCjunior.
2. Select **Help|Help Topics** from the main screen.
3. Double-click on **Getting Started.**
4. Double-click on **Guided Tour.**
5. Double-click on **Guided Tour,** then follow the directions.

   If the Help window is in your way, you can **move** and **resize** it as you take the tour. It will stay "on top" of KCjunior.

Prepare the Reader

Refer to the reader's user manual for assistance if necessary.

1. Connect the reader to the computer and turn it on.
2. Through the reader's front panel, find the **Baud Rate, Data Bits, Stop Bits,** and **Parity** information.
3. Note the current settings for each parameter. Make the following changes if necessary:
   - If the **Baud Rate** is currently set to 1200 bps, change it to at least 2400 bps.
   - If **Data Bits** is currently set to 7, change it to 8.
4. Return the reader front panel display to the main menu.
5. Perform a reader system test if any changes were made.

Define and Test Communication Parameters

It is important to set and test communication parameters *before* attempting to read a microplate.

1. Start KCjunior, if it is not already loaded.
2. In KCjunior, select **Setup|Reader.**
3. Select the appropriate **Reader Type.**
4. Select the appropriate **Com Port** setting.
5. Enter **Baud Rate, Data Bits, Parity,** and **Stop Bits** values to match those on the reader.
6. Click the **Test Communications** button.
7. If the test fails, follow the directions provided by KCjunior, then click **Test Communications** again.
8. If the test passes, click **OK** to save the settings and close the dialog.

Create a Protocol

A protocol contains instructions for controlling the reader and for analyzing data retrieved from the reader.

1. Click the **New Protocol** button in the main screen.
2. Enter a **Protocol Name** using up to 16 alphanumeric characters.
3. Set the **Raw Data Limits** Minimum and Maximum Values to -3.000 and 3.000, respectively.
4. Set the **Blank Limits** Minimum and Maximum Values to -1.000 and 1.000, respectively.
5. Leave all other parameters set to their default values.
6. Click the **Read Method** tab.

Define Read Method Parameters

The configuration of the **Read Method** parameters controls plate reading.

1. Click the **Get Reader Wavelengths** button to retrieve wavelength settings from the reader.
2. Select a **Read Method** of Endpoint.
3. Select any **Primary Wavelength.**
4. Leave all other parameters set to their default values.
5. Click the **Template** tab.

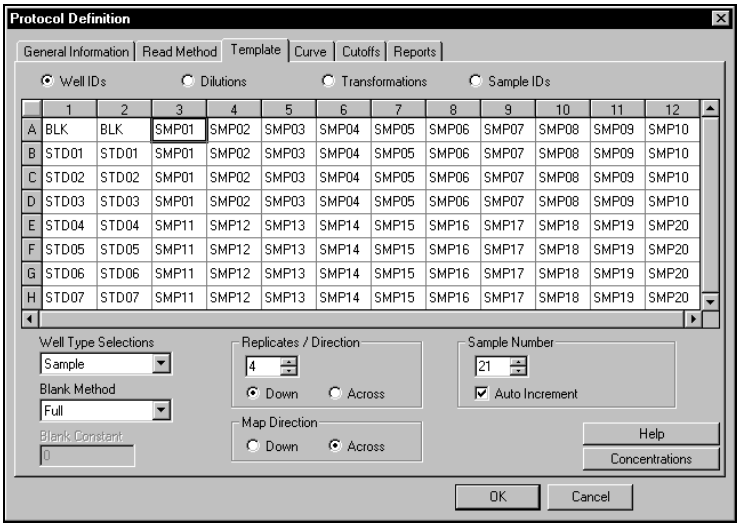
Design Templates

In each KCjunior protocol, four unique Templates can be designed to indicate location and related information for **well** and **sample identifiers, dilution values,** and **transformations.**

Well IDs

1. Select "Blank" from the **Well Type Selections** list, then click on wells **A1** and **A2.** Leave **Blank Method** set to **Full.**
2. Select "Standard" from the **Well Type Selections** list.
3. Set **Replicates/Direction** to **2** and **Across,** respectively.
4. Click and hold the cursor on well **B1.** Drag the cursor to well **H2** and release. Seven groups of Standards should be assigned in duplicate.
5. Select "Sample" from the **Well Type Selections** list.
6. Set **Replicates/Direction** to **4** and **Down,** respectively.
7. Set **Map Direction** to **Across.**
8. Click and hold the cursor on well **A3.** Drag the cursor to well **H12** and release. Twenty groups of Samples should be assigned in quadruplicate.

   The Well ID Template should look like this:



	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	BLK	SMP01	SMP02	SMP03	SMP04	SMP05	SMP06	SMP07	SMP08	SMP09	SMP10
B	STD01	STD01	SMP01	SMP02	SMP03	SMP04	SMP05	SMP06	SMP07	SMP08	SMP09	SMP10
C	STD02	STD02	SMP01	SMP02	SMP03	SMP04	SMP05	SMP06	SMP07	SMP08	SMP09	SMP10
D	STD03	STD03	SMP01	SMP02	SMP03	SMP04	SMP05	SMP06	SMP07	SMP08	SMP09	SMP10
E	STD04	STD04	SMP11	SMP12	SMP13	SMP14	SMP15	SMP16	SMP17	SMP18	SMP19	SMP20
F	STD05	STD05	SMP11	SMP12	SMP13	SMP14	SMP15	SMP16	SMP17	SMP18	SMP19	SMP20
G	STD06	STD06	SMP11	SMP12	SMP13	SMP14	SMP15	SMP16	SMP17	SMP18	SMP19	SMP20
H	STD07	STD07	SMP11	SMP12	SMP13	SMP14	SMP15	SMP16	SMP17	SMP18	SMP19	SMP20

   - Clear any mistakes by selecting **<Clear>** from **Well Type Selections** and highlighting the well(s) that need to be changed.

9. Click the **Concentrations** button.

Concentrations

Concentrations can be assigned from within either the Well ID template or the Curve dialog.

Here you will assign a concentration value to each standard group.

1. Set the starting **Concentration** to 1.000.
2. Click **Factor,** and set the **Factor Value** to **2.**
3. Click the title cell of the **CONC** column. Concentrations should be assigned as 1, 2, 4, 8, 16, 32, and 64.
4. Click **OK.**
5. Click the **Dilutions** radio button.

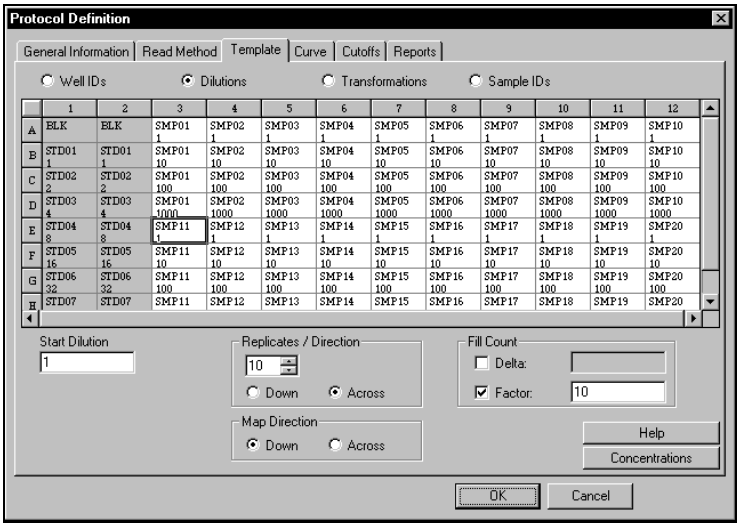
Dilutions

In quantitative assays, calculated concentrations are multiplied by the dilution values to produce the final concentrations.

Here you will assign dilutions for each sample group, increasing by a factor of 2.

1. The starting **Dilution** should be set to 1.000 by default.
2. Set the **Replicates/Direction** to **10** and **Across,** respectively.
3. Click **Factor,** and set the **Factor Value** to **10.**
4. Set **Map Direction** to Down.
5. Click and hold the cursor on well **A3.** Drag the cursor to **D12** and release.
6. Set the starting **Dilution** back to **1.000.**
7. Click and hold the cursor on well **E3.** Drag the cursor to **H12** and release.

   The Dilutions Template should look like the one shown on the next page.



8. Click the **Transformations** radio button.

Transformations

Two types of transformations can be defined within one protocol:

- A **Transformation Formula** is performed only on the well(s) to which it is assigned.
- A **Plate Formula** is automatically applied to every well on the plate.

Here you will apply **Transformation Formulas** to subtract the Blanked OD value from each well in column 4 from its corresponding well in column 3.

Note: KCjunior automatically calculates formulas based on the **Blanked Data** if Blank wells are defined in the Well ID Template. If no Blank wells are defined, formulas are based on the **Raw Data.**

1. In the **Transformation Formula** field, enter **X-[A$4].**

   X = current well

   - = subtraction

   A4 = contents of well A4

   [ ] indicates a well reference

   $ retains the column number 4 when copying the formula

2. Highlight well **A3,** then click the **Apply** button. The formula should appear under SMP01 in well A3.
3. With the cursor on A3, click the **right** mouse button. An editing menu should appear.
4. Select **Copy** from the editing menu.

5. Click and hold the cursor (use the **left** mouse button) on well **B3.** Drag the cursor to well **H3** and release. Wells B3 to H3 should now be highlighted.
6. Click the **right** mouse button to open the editing menu, then click **Paste.**

   Well B3 should contain X-[B$4], well C3 should contain X-[C$4], and so on to H3, X-[H$4].
7. Click the **Sample IDs** radio button to continue.

Sample IDs

By default, KCjunior refers to sample groups as "SMP01", "SMP02", "SMP03", and so on.

Within the Sample ID template, you can assign **custom identifiers** to sample groups.

1. Click on any well in Sample group number 1. "SMP01" will appear in the **Sample ID** field.
2. Overwrite "SMP01" with any name in the **Sample ID** field, then click the **Apply** button. The ID will be assigned to every sample in group 1.
3. Repeat for several more samples.
4. Click the **Curve** tab.

Define Curve Parameters

KCjunior provides three methods for generating curves:

- A **standard curve** can be generated based on the standard wells defined within the protocol.
- A previously generated and **stored curve** can be referenced from within the protocol.
- A **titer curve** can be generated using multiple dilutions of sample groups as concentrations.

Here you will define parameters to generate a point-to-point standard curve.

1. Select the **Point-to-Point** curve method.
2. Check **Extrapolate** to display extrapolated concentrations – unknown values on the Y axis which are outside the range of the standards.
3. Leave all other parameters set to their default values.
4. Click the **Cutoffs** tab.

Define Cutoffs

User-defined **Cutoffs** are used to classify results. During data reduction, results are evaluated against the cutoff criteria and each well is assigned a user-defined **Label.**

Here you will create three cutoff "zones":

1. Enter "STD01" in the first **Cutoff** field.
2. Enter "STD04" in the second **Cutoff** field.
3. Enter "Neg" in the first **Label** field, "Eqv" in the second, and "Pos" in the third.
4. Click the **Reports** tab.

Select Reports

The **Reports** dialog is used to select and customize reports for this protocol.

1. Enter any text in the **Report Banner** field.
2. Check the **Absorbance Report** box and the accompanying **Both** radio button.
3. Check the **Matrix Report** box.
4. In the **Options** list, highlight **Well IDs,** then click **Add.**
5. In the **Options** list, highlight **Calculated Concs,** then click **Add.**
6. Check the **Curve Report** box.
7. Click **OK** to close the protocol.
8. Select **Protocol|Save Protocol.**

Read a Plate

1. Click the **Read Plate** button.
2. Enter a **Results ID,** using up to 16 alphanumeric characters.
3. Click **Read Plate.**
4. Place a microplate on the reader.

   Note: For best results:

   - Wells **A1** and **A2** should be empty.
   - The contents of wells **B1** and **B2** should result in a low optical density value at the defined Primary Wavelength.
   - **C1** and **C2** should read slightly higher, and so on through **H1** and **H2** which should provide the highest OD.
5. Click **OK.**

Interpret Results

Following a successful plate read, **Raw Data, Blanked Data, Transformation Results, Concentrations, Labels** and **Template** information will be displayed.

1. Click on each of the six tabs to see results.
2. Under **Concentrations,** click **Standard Curve** to see the standard curve.
3. Under any tab, click **Statistics** to see statistical information for every Standard, Sample, and Blank group.

Print Reports and Store Results

1. If a printer is connected to the computer, either directly or via a network, select **Results|Print Results** to print reports.
2. To save the results record, select **Results|Save Results.** The record will be stored under the **Results ID** in the **Results Database.**

Setting Up the System

Reader Support

KCjunior supports most of Bio-Tek's automated microplate readers, including:

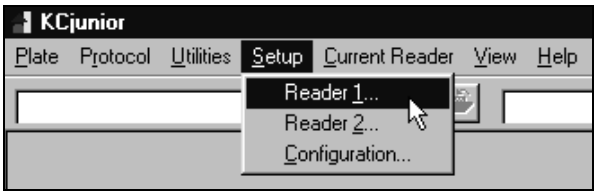
EL301	ELx800	PowerWave 200
EL311	EL808	PowerWave 340
EL312	ELx808	PowerWaveX
EL340	FLx800	PowerWaveX 340
EL800	µQuant	PowerWaveX Select
		PowerWave XS

Dual-Reader Support

KCjunior can be configured to communicate with any **two** Bio-Tek readers, if the computer has two serial ports. This allows you to, for example, connect an absorbance reader and a fluorescence reader to one computer and switch easily between them from within KCjunior.

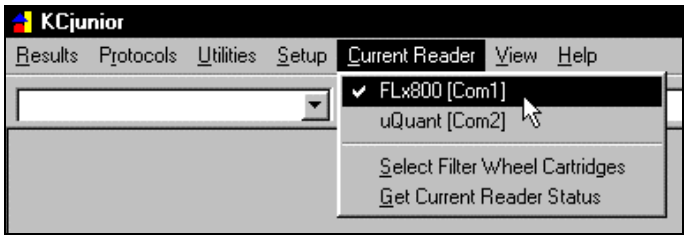
Note: If you have a laptop computer, you may need to install a communications card.

- To configure KCjunior's communication parameters, start by selecting **Reader 1** or **Reader 2** from the Setup Menu, as shown below.



➢ See "Reader Setup" on page 4-3 for further instructions.

- To switch between the two readers, use the **Current Reader Menu.**



  - ➢ KCjunior automatically switches readers if a protocol is opened that is not compatible with the currently selected reader.

Connecting the Reader to the Computer

Before KCjunior can communicate with a microplate reader, the reader(s) must be properly connected to the computer on which KCjunior is installed.

The following steps briefly describe how to connect the reader to the computer. For more detailed information, refer to the reader's user manual.

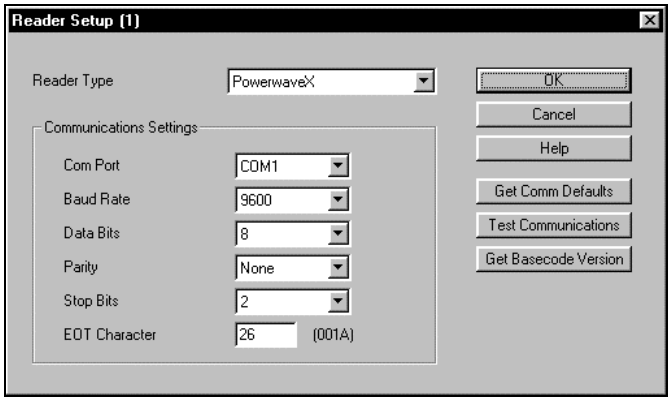
1. Attach the appropriate cable to an available serial port on the back of the computer.
   - ➢ If you are going to configure KCjunior for use with two readers, the computer must have two serial ports.
2. Plug the other end of the cable to the serial port on the reader.
3. Connect the power cable to the back or side of the reader.
4. Plug all power cords into grounded electrical outlets.
   - ➢ Use of a surge protector is strongly recommended.
5. Use KCjunior to set up the communication parameters, as described in the next section, **Reader Setup.**

Reader Setup

Important! Set and test communication parameters *before* attempting to read a microplate. Follow these steps in order:

1. Ensure that the microplate reader is connected to the computer on which KCjunior is loaded, as described in the previous section.
2. From the reader's front panel (if the reader is equipped with one), find the **Baud Rate, Data Bits, Stop Bits,** and **Parity** information. Refer to the reader's user manual for assistance if necessary.
3. Note the current settings for each parameter. Make the following changes if necessary:
   - If the **Baud Rate** is currently set to 1200, change it to at least 2400.
   - If **Data Bits** (or Char Bits) is currently set to 7, change it to 8.
4. Perform a reader system test if any changes were made above.
5. **Important!** Return the reader's display to the main menu.
6. Start KCjunior.
7. Within KCjunior's main screen, select **Setup|Reader 1** or **Reader 2.** The **Reader Setup** dialog appears:

*For communication parameter changes to take effect on an **EL311,** the reader must be turned off/on after any changes are made.*



*Select the appropriate Reader Type **before defining protocols,** even if the reader is not actually connected to the computer.*

*Click **Get Basecode Version** to retrieve the attached reader's basecode software part number and version number.*

8. Select a **Reader Type** from the drop-down list.
   - **Choose carefully** -- it is important to select the correct model. For example, be sure to select "ELx808I" for an ELx808 with the incubation option.
   - Select "PowerwaveX" for any **PowerWaveX** model other than the PowerWave XS. Do *not* select "PowerWave340" or "PowerWave340I" for a PowerWave**X** 340 instrument.

9. (Optional) Click the **Get Comm Defaults** button to set the communication parameters to the default values associated with the selected **Reader Type.**
   - If the current communication settings are acceptable, jump ahead to step 16.
10. Select a **Com Port.** The Com Port is the computer's communication port that is used for data transmission between KCjunior and the reader.
    - Options include COM1, COM2, COM3, and COM4.
11. Select a **Baud Rate.** The Baud Rate is the transmission speed of data, in bits per second, between KCjunior and the reader.
    - Options include 1200 (slow), 2400, 4800, 9600, 19200, and 38400 (fast). Valid settings vary among readers.
12. Set the **Data Bits** value to 8.
13. Select a **Parity** setting. Parity is a method of checking for errors during data transmission.
    - Options include None, Odd, and Even.
14. Set the **Stop Bits** value to 2.
15. Specify an **EOT Character.** This represents the character sent by the reader to KCjunior to indicate the end of transmission of a plate of data.
    - The default setting is **26** (001A, ^Z).
    - For the **EL311, EL312,** and **EL340** readers, the EOT Character may be changed. The setting in KCjunior must match that on the reader.
    - For the **EL301** microstrip reader, the EOT Character has no significance.
    - The **FLx800** does not require an EOT Character.
    - For all other readers, the EOT Character must be set to **26.**

If the EL301 is the current reader, clicking Test Communications only verifies the parameter settings. KCjunior will not attempt to communicate with the EL301.

16. When all parameters are set, click the **Test Communications** button to attempt to establish communication with the reader.
    - If the test is *successful*, the communication parameters are set correctly.
    - If the test is *unsuccessful*, appropriate instructions are provided for fixing the communication parameters.
17. Click **OK** to save the settings, close the **Reader Setup** dialog, and return to the KCjunior main screen.

System Configuration

Various system default parameters can be configured within the **Setup|Configuration** group of dialogs.

Configurable parameters include well identifiers, several reporting options, results and protocol database pathnames, and well type colors.

Well Identifiers

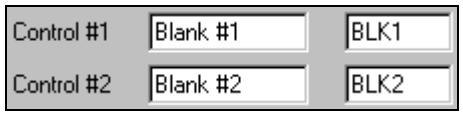
KCjunior supports four different types of wells – **Blank, Standard, Sample,** and **Control**, and processes each type as follows:

- The average of the **Blank** wells is automatically subtracted from every well on the plate.
- The values in the **Standard** wells are used to generate a standard curve.
- KCjunior recognizes individual **Sample** wells and sample *groups* (if multiple replicates are defined).

  The mean, standard deviation, and coefficient of variation are automatically calculated for each Sample group. If defined in the protocol, concentrations and cutoffs are calculated for each Sample replicate and group.

  If dilutions are defined, KCjunior generates a curve for each sample group.
- Up to five unique sets of **Control** wells can be defined (see "Customizing Well IDs" on the next page).

  This well type can be used to represent a typical control category (e.g. positive control, negative control), or to fulfill a special requirement that the Blank, Standard, and Sample well types do not satisfy. For example, if an assay requires two unique Blank well identifiers, define two sets of Control wells as follows:



  Note: To perform blank subtraction for an assay using the Well IDs shown above, a transformation formula resembling **X-BLK1** must be assigned to every well from which the BLK1 mean will be subtracted, and **X-BLK2** must be assigned to every well from which the BLK2 mean will be subtracted. See "Working with Transformation Formulas" in *Defining Protocols* for more information.

Customizing Well IDs

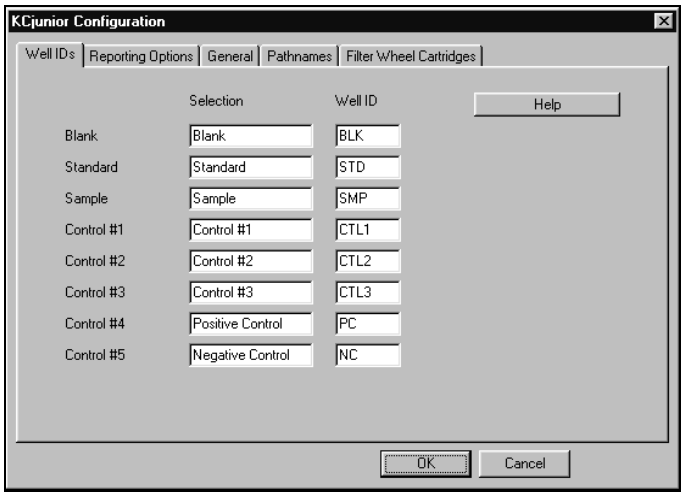
*Well Identifiers are assigned to microplate wells within the **Well ID Template.** See "Designing a Well ID Template" in **Defining Protocols** for more information.*

To accommodate a variety of applications, KCjunior provides the flexibility to customize references to the different well types. These customized references are called **Well Identifiers.**

- For example, a well that KCjunior consistently treats as a Standard behind-the-scenes can be identified as "STD01" or "STDRD01" or "CAL03" in a Well ID Template, in results displays, and on printed reports.

To configure **Well Identifiers:**

1. Select **Setup|Configuration.** The **Well IDs** dialog appears:



2. Enter a **Selection** name and its associated **Well ID** for each default well identifier.

   - The **Selection** name is the descriptive link between the generic well type (i.e., Blank, Standard, Sample, Control), and the 2 to 5 character Well ID.

     Each Selection can contain up to 16 alphanumeric characters, including spaces.

   - The **Well ID** is the short identifier that appears in results displays and on printed reports. It can also be used in transformation, plate, and cutoff formulas.

     Each Well ID can contain 2 to 5 alphanumeric characters, with **no** spaces.

     Note: Changes to default Well IDs may affect future modifications to existing Well ID templates. See "Modifying an Existing Well ID Template" in *Defining Protocols* for more information.

➢ **Usage example:** If an assay requires the use of Calibrators to generate the standard curve, set the **Selection** name and **Well ID** for Standard wells to "Calibrator" and "CAL", respectively, as shown below:

With these settings, KCjunior generates the standard curve based on the ODs measured in the wells assigned as "Calibrator".

For more information, see "Designing Templates - Well ID Templates" in *Defining Protocols.*

3. Click another tab to configure additional system parameters, or click **OK** to save these settings, close the dialog, and return to the KCjunior main screen.

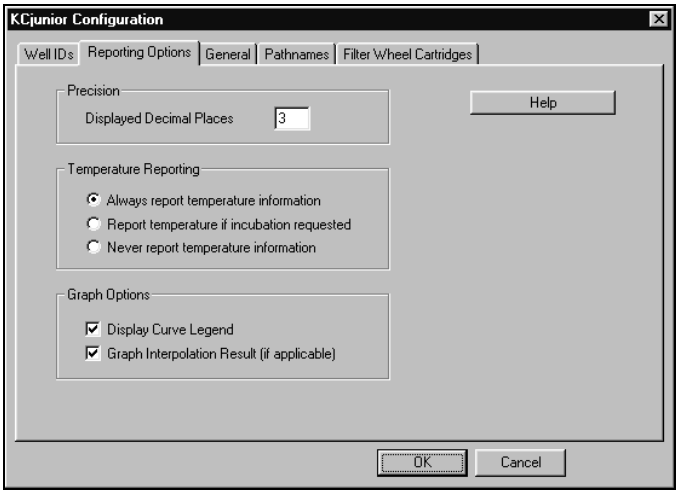
Reporting Options

KCjunior provides several configurable reporting options, including precision, reader temperature, and special graph options.

To configure **Reporting Options:**

1. Select **Setup|Configuration.** Click the **Reporting Options** tab.

   The **Reporting Options** dialog appears:



2. Set the **Displayed Decimal Places** value. Values such as optical density measurements, transformation results, and concentrations are reported with the number of decimal places defined in this field.

   ➢ The range is from 0 to 3 decimal places.

3. If the reader supports incubation, select a **Temperature Reporting** option for printed reports:
   - Select **Always report temperature information** to report the reader temperature even if a plate is read with a protocol that does not specify incubation.
   - Select **Report temperature if incubation requested** to report the reader temperature only when a plate is read with a protocol that specifies incubation.
   - Select **Never report temperature information** to suppress all temperature information.
4. Specify **Graph Options:**
   - Check **Display Curve Legend** to include a legend on the curve display and report.
   - Check **Graph Interpolation Result** to plot the interpolation value on the curve display and report.
5. Click another tab to configure additional system parameters, or click **OK** to save the change, close the dialog and return to the KCjunior main screen.

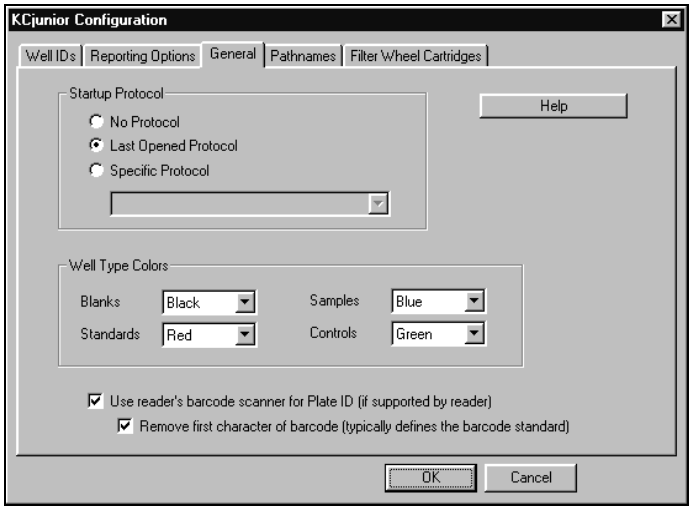
General Default Information

KCjunior provides several configurable general system parameters, including **Startup Protocol, Well Type Colors,** and **Use Reader's Barcode Scanner for Plate ID.**

To configure **General Default Information:**

1. Select **Setup|Configuration.** Click the **General** tab.

   The **General** dialog appears:



2. Specify a **Startup Protocol.** Instruct KCjunior to automatically open the most recently opened protocol, a specific protocol, or no protocol every time the software is started.
   - If **Specific Protocol** is selected, choose a protocol from the drop-down list.
3. Change the **Well Type Colors.** To differentiate between the four well types, each can be set to a different color.
   - Available colors are Red, Blue, Green, Yellow, Magenta, Light Blue, Black, Light Gray, and Dark Gray.
4. If the attached reader has a barcode scanner, enable **Use Reader's Barcode Scanner for Plate ID** to scan the barcode label on the plate before it is read.
   - If this option is enabled, KCjunior attempts to scan the barcode when the plate is pulled into the reader. If the read is unsuccessful, KCjunior then asks the operator to enter the Plate ID manually.
   - The scanned or manually entered Plate ID is available as a part of the report/export header information.
   - This option is supported for single and multi-plate reads. It is not supported for the empty plate(s) of a Multi-Wavelength read.
   - If the barcode returned from the reader is preceded by an unnecessary identification character, you can check **Remove first character of barcode** to have KCjunior remove it.
5. Click another tab to configure additional system parameters, or click **OK** to save the change, close the dialog and return to the KCjunior main screen.

Pathnames

Results records and protocols are stored in designated **Results** and **Protocol Databases,** respectively. When opening or closing results records and protocols, KCjunior automatically refers to the Results and Protocol Database **Pathnames** for retrieval and storage.

- Select **Setup|Configuration, Pathnames** to see the current configurations.

Important!

If a database's location changes, the pathname must be reconfigured.

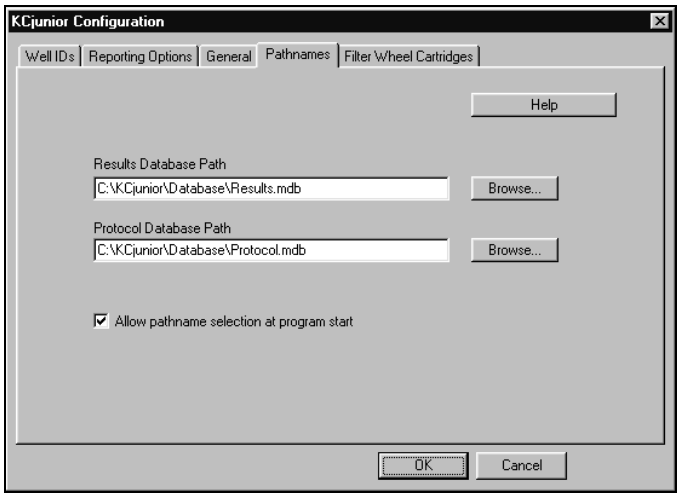
The default results and protocol databases are named **RESULTS.MDB** and **PROTOCOL.MDB,** respectively. These databases can be copied, moved, or renamed using any file management software.

- For example, the protocol database may be moved to a network to be shared by multiple users and renamed as NTWRKPRT.MDB.

To **reconfigure** the Results or Protocol Database Pathname:

1. Close any open results records and protocols.
2. Select **Setup|Configuration.** Click the **Pathnames** tab.

   The **Pathnames** dialog appears:



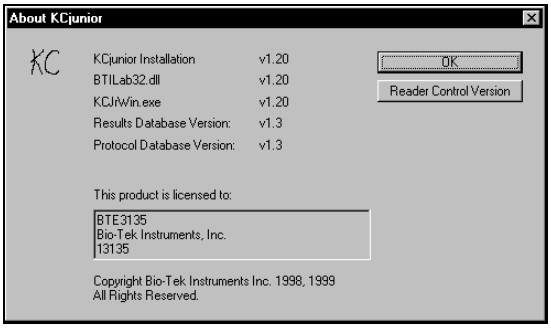
3. In the **Results Database Path** or **Protocol Database Path** field, enter the new location and/or name manually, or use the **Browse** button to search through the directory structure to locate the desired database.
4. (Optional) KCjunior provides an option to present the Pathnames dialog each time the software is loaded, allowing the user to change the database pathname(s). To enable this option, check **Allow Pathname Selection at Program Start.**
5. Click another tab to configure additional system parameters, or click **OK** to save the changes, close the dialog and return to the main screen.

Database Version

A small number of KCjunior features, such as Spectral Scanning, user-enabled Scientific Notation, and Pathlength Correction, require the protocol and/or results database to be at or above a certain **version number.**

- This is only a concern if databases were created with an earlier version of KCjunior. A software upgrade *may* contain new features that require newer databases. The **User's Guide** and **Help System** show the minimum required database version number next to each feature that has such a requirement.

- To see the version numbers of the currently configured protocol and results databases, select **Help|About KCjunior.**



  - Protocols and results records from an "older" database can be **imported** into a newer database with a higher version number.

    See "Importing Protocols" in *Defining Protocols* for more information.

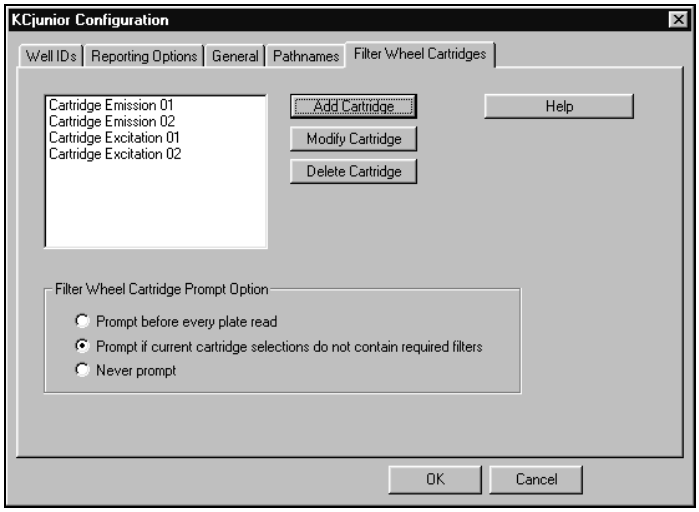
Filter Wheel Cartridges

If one or both of the defined readers is the FLx800 (a fluorescence/ luminescence reader), **Filter Wheel Cartridges** is available within the Setup|Configuration set of tabs.

The FLx800 has one slot for a removable **Excitation** filter wheel cartridge, and another for a removable **Emission** filter wheel cartridge. Each cartridge has four locations for filters or plugs. A location can also be left "open" (no filter or plug).

For a FLx800 that is used for a variety of applications, multiple Excitation and/or Emission filter wheel cartridges may be prepared and installed as needed. It is important for KCjunior to know the exact values and positions of the filters/plugs/holes for the cartridge currently in use. To simplify the filter definition process when multiple Excitation and/or Emission filter wheel cartridges are used, the contents of each cartridge can be entered once into KCjunior and given a name such as "Cartridge Excitation 01". When changing to a different cartridge, simply select its assigned name in KCjunior's **Current Reader Menu** to prepare to create protocols and read plates.

To add, modify, or delete a filter wheel cartridge configuration, select **Setup|Configuration.** Click the **Filter Wheel Cartridges** tab:

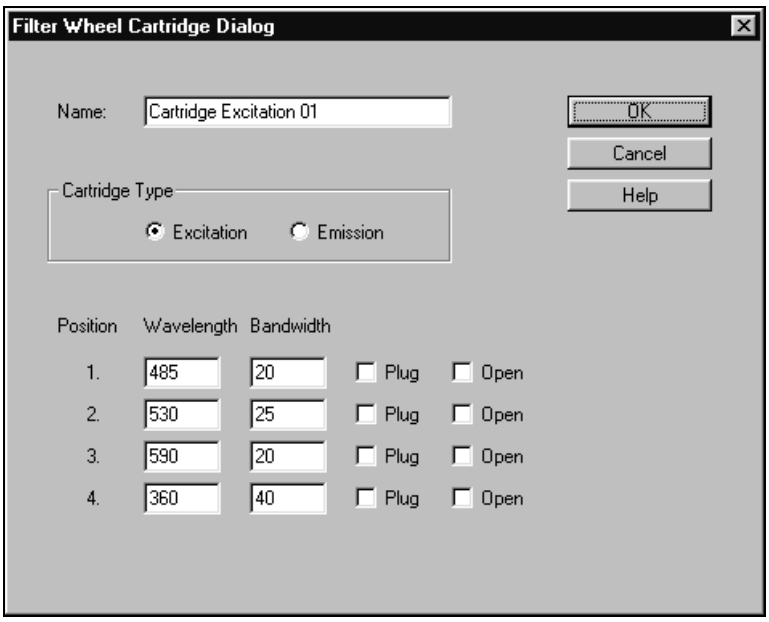


- Click **Add Cartridge** to configure settings for a new filter wheel cartridge and add it to the list.
- Select a cartridge name then click **Modify Cartridge** to modify settings for an existing filter wheel cartridge.
- Select a cartridge name then click **Delete Cartridge** to permanently delete the cartridge from the list.
- KCjunior can be configured to present a **prompt** just prior to a plate read, to allow the operator to specify which filter cartridges are installed on the instrument. When a new cartridge is selected, KCjunior updates the instrument with the new filter information.
  - Choose **Prompt before every plate read** to present the prompt before every FLx800 plate read.
  - Choose **Prompt if current cartridge selections do not contain required filters** to present the prompt only if there is a mismatch between the protocol and the instrument's filter table.
  - Choose **Never prompt** to prevent the prompt from appearing. **Note:** If there is a mismatch between the protocol and the instrument's filter table, KCjunior presents an appropriate message.

Adding or Modifying a Filter Wheel Cartridge

From within the Filter Wheel Cartridges tab, click **Add Cartridge** to configure a new cartridge, or select an existing cartridge name and click **Modify Cartridge.**

The **Filter Wheel Cartridge Dialog** appears:



Define the characteristics for this filter wheel cartridge.

1. Enter a unique **Name** for the cartridge, using up to 32 alphanumeric characters.
2. Select the **Cartridge Type,** Excitation or Emission.
3. For each filter wheel position, enter the **Wavelength** and **Bandwidth** information, or select **Plug** or **Open.**
   - It is important that the wavelength and bandwidth information be entered correctly for each filter. The wavelength and bandwidth values are often printed on the side of each filter.
   - **Dispense / Read** protocols have special filter position requirements; turn to the next page for more information.
4. Click **OK** to save the changes and return to the previous screen, or click **Cancel** to discard the changes.

Special Filter Position Requirements for Dispense / Read Protocols

Single filter set protocols:

- If light shuttering is specified, a blocking filter (Plug) must be placed in the excitation filter wheel in one of the two positions next to the excitation filter that is specified in the protocol.

Dual filter set protocols:

- The two excitation filters specified in the protocol must be positioned next to each other in the excitation filter wheel.
- The two emission filters specified in the protocol must be positioned next to each other in the emission filter wheel.
- If light shuttering is specified, two blocking filters (Plugs) must be placed in the excitation filter wheel.

Defining Protocols

Overview

A **Protocol** contains:

- Instructions for controlling the attached microplate reader.
- Information for analyzing data retrieved from the reader (or for analyzing data entered manually or read from a file).

User-definable protocol **features** include:

- Protocol name and description
- Minimum and maximum optical density limits
- Minimum and maximum relative fluorescence/luminescence unit limits
- Reading method (Endpoint, Kinetic, MultiWavelength, Spectral Scan, or Dispense / Read), and related parameters
- Fluorescence/Luminescence-specific parameters such as chemistry, number of filter sets, optics position, and sensitivity
- Template design for :
  - Well IDs
  - Dilutions
  - Transformation and Plate Formulas
  - Sample IDs
- Curve-fitting parameters
- Cutoff criteria
- Reporting options

Defining Protocols describes how to customize the properties of every feature listed above.

Working with Protocols

This section provides information for creating, changing, accessing, storing, and sharing protocols.

Where are Protocols Stored?

Protocols are stored inside a **Protocol Database,** which is a file that is typically located on a computer's hard drive, or on a network to which the computer is attached.

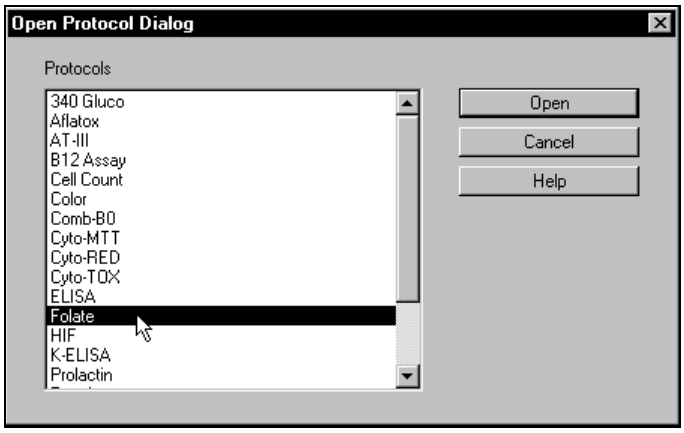
When opening or saving protocols, KCjunior automatically refers to a user-specified Protocol Database Pathname for retrieval and storage.

- The **Protocol Database Pathname** contains the name and location of the "current" protocol database. Multiple protocol databases can be stored on a computer or on a network, but there can only be one *current* database.
  - Example pathname: C:\KCjunior\Database\Protocol.mdb.
- Select **Setup|Configuration, Pathnames** to view or modify the current protocol database pathname. See "Pathnames" in *Setting Up the System* for more information.

Opening an Existing Protocol

To open an existing protocol, begin by clicking the **Open Protocol** button in the KCjunior main screen, selecting **Open Protocol** from the Protocol Menu, or by selecting a name from the protocol drop-down menu in the toolbar.

The **Open Protocol** dialog appears:



- This dialog lists the names of all protocols available in the currently configured **Protocol Database.**
- To open a protocol, highlight the desired name in the list, then double-click the mouse button, or click the **Open** button.

➢ If KCjunior is configured for use with two readers:

When a protocol is opened, KCjunior attempts to determine which reader best supports the protocol, and then sets it as the **Current Reader** (see "Reader Support" in *Setting Up the System* for more information). Selection criteria includes fluorescence / absorbance reading capabilities, and read method parameters. If the readers are equally capable of supporting the protocol, KCjunior does not change the Current Reader.

- To close this dialog without opening a protocol, click **Cancel** or press the **Esc** key.
- To select a different **Protocol Database,** close this dialog then select Setup|Configuration|Pathnames.

Creating a New Protocol

To create a new protocol, begin by clicking the **New Protocol** button in the KCjunior main screen, selecting **New Protocol** from the Protocol Menu, or by clicking the **toolbar icon.**

The **General Information** dialog appears, requesting identification information for the new protocol. See "Defining General Information" at the start of *Defining Protocol Parameters* for more information.

Before Defining a New Protocol

Set the appropriate Reader Type before defining a protocol. See "Reader Setup" in ***Getting Started*** *for more information.*

The available, definable parameters for every new protocol vary depending on the currently configured **Reader Type.** Note: Before defining a new protocol, make sure the Reader Type is properly set under **Setup|Reader,** and the correct reader is selected under **Current Reader.**

Examples:

- If the currently selected Reader Type is **ELx808,** the available Read Method Types are *Endpoint* and *Kinetic.*
- If the currently selected Reader Type is **PowerWave 340,** the available Read Method Types are *Endpoint*, *Kinetic*, and *MultiWavelength*, and the available wavelength range is limited to 340-800 nm.

Modifying Protocols

To modify an existing protocol, begin by opening the desired protocol (select Protocols|Open Protocol).

Once the protocol is open, click the **Modify Protocol** button in the KCjunior main screen, select **Modify Current Protocol** from the Protocol Menu, or click the **toolbar icon.**

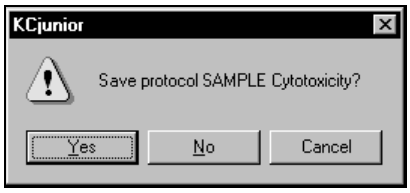
The **General Information** dialog appears, containing identification information for that protocol. See "General Information" at the start of *Defining Protocol Parameters* for more information.

Saving and Closing Protocols

After creating or modifying a protocol, *save* the information by selecting **Save Protocol** from the Protocol Menu or by clicking the toolbar icon.

- KCjunior stores the protocol in the current protocol database under the name defined in the **General Information** dialog.

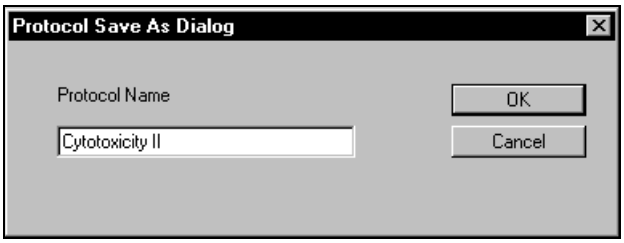
To *close* an open protocol, select **Close Protocol** from the Protocol Menu, or click the toolbar icon. If the protocol is newly created, or if modifications were made to an existing protocol, KCjunior provides an option to save:



- Click **Yes** to save the new or modified information and close the protocol.
- Click **No** to close the protocol without saving changes.
- Click **Cancel** to close this dialog and return to the open protocol. The new or modified information is retained but not yet saved.

Copying Protocols

To copy the contents of an existing protocol to a new one with a different name, begin by opening the protocol to be copied. Once the protocol is open, select **Save As** from the Protocol Menu. KCjunior asks for a new protocol name:



- Enter a new protocol name using up to 32 alphanumeric characters and spaces, then click **OK.** The new protocol is created, saved, and kept open for modification.
- Click **Cancel** to cancel this operation and return to the original protocol.

Sharing Protocols and Databases

Since KCjunior stores protocols in a database, there are several options for **sharing** protocols and protocol databases among users:

- A protocol database can be stored on a **network** for multi-user availability.
  - Each user must configure the **Protocol Database Pathname** within his or her copy of KCjunior to point to the network database.
- A protocol database can be copied to a diskette or CD and exchanged between users.
  - If the database has grown too large to fit on a diskette, create an empty protocol database and then copy only the desired protocols into the new database:
    1. Select **Utilities|Database Maintenance|Create Archive Protocol Database.** The **Create Empty Protocol Database** dialog appears.
    2. In the **Save in** field, select a location for the new database, then enter an identifying **File** name.
    3. Click **Save** to create the database and return to the main screen.
    4. Select **Utilities|Database Maintenance|Archive Protocols.** The **Archive Protocol Database Pathname** dialog appears.

5. Enter the drive, directory, and file name for the newly created empty database.
6. Click **OK.** The **Archive Protocols** dialog appears, listing the contents of the current Protocol Database.
7. Select the protocol(s) to be copied. Do **NOT** check **Delete after archive.**
8. When all items have been selected, click the **Archive Protocol(s)** button. The selected items are copied to the new database.

- Protocols can be **imported** from one database into another. See "Importing Protocols" below for more information.

  Suggestions:

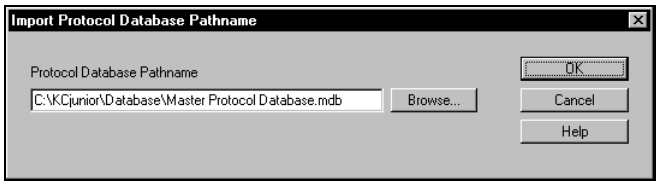
  - Protocols can be imported from a "master" protocol database stored on a network.
  - Protocols can be imported from a protocol database stored on a diskette or on a CD.

Importing Protocols

Protocols from one Protocol Database can be **imported** into another. The Protocol Database that will *receive* the imported protocols is the one defined under **Setup|Configuration|Pathnames.**

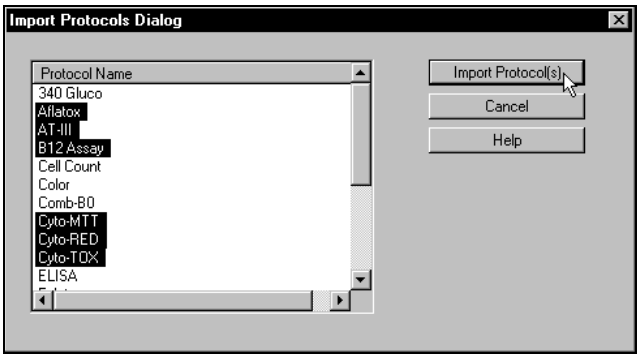
To import protocols from another database:

1. If a protocol is open, close it.
2. Select **Protocol|Import Protocols.** The **Import Protocol Database Pathname** dialog appears.



3. In the **Protocol Database Pathname** field, enter the pathname (drive, directory, and file name), of the database containing the protocols to be imported.

   Alternatively, use the **Browse** feature to locate and select the database.
4. Click **OK.** The **Import Protocols** dialog appears, listing all protocols in the database.



5. Use the mouse to **select** the protocol(s) to be imported.
   - Press the **Shift** key while using the mouse to select contiguous protocols.
   - Press the **Ctrl** key while using the mouse to select non-contiguous protocols.
6. Click the **Import Protocol(s)** button. The protocol import begins.
   - If a duplicate **Protocol Name** is detected, KCjunior does *not* overwrite the protocol.

Defining Protocol Parameters

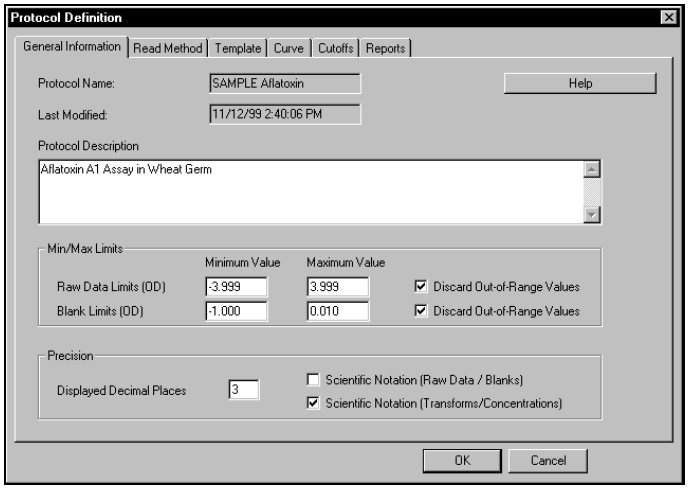
When creating a new protocol, a minimum set of **General Information** and **Read Method** parameters must be defined for the protocol to be "valid". In addition, **Templates, Curve** parameters, **Cutoff** criteria, and **Report** options can be specified. *Defining Protocol Parameters* describes every step of the protocol definition process.

Defining General Information

General Information includes protocol identification, minimum/maximum limit setting, and precision options.

To define this information for a protocol:

1. Choose to create or modify a protocol. Select the **General Information** tab. The General Information dialog appears:



2. If this is a new protocol, enter a **Protocol Name,** using up to 32 alphanumeric characters. The protocol will be stored in the current protocol database under this name.
3. (Optional) Enter a **Protocol Description,** using up to 1024 alphanumeric characters.
   - Text entered here appears at the top of the **Protocol Report.**
4. (Optional) Adjust the **Raw Data Limits** Minimum and Maximum Values to define an inclusive data range for **all wells.** The valid limits are reader-dependent.

*The **Raw Data Limits (OD) Minimum Value** can be set to a number less than zero, to accommodate dual wavelength reads.*

For example, if the Minimum Value is set to -0.500 OD, a post-dual wavelength subtraction value of -0.600 OD falls outside the valid range

Explanation:

During data reduction, the OD, RFU, or RLU value in each well is verified against this range. If the value falls outside the range, two options are available:

- **Discard the out-of-range value.** If Discard Out-of-Range Values is selected, the well is reported as **OUT** and is excluded from data reduction calculations. For example, if one replicate of a sample group is reported as **OUT,** the sample group mean is based on the remaining in-range replicates.
- **Retain the out-of-range value.** If Discard Out-of-Range Values is **not** selected, KCjunior automatically sets the raw OD, RFU, or RLU value to the Minimum or Maximum Raw Data Limits value, as appropriate. The new value is displayed inside brackets **[**2.999**]**, and is included in further calculations (such as group mean calculation, transformations, and standard curve generation).

5. (Optional) Adjust the **Blank Limits** Minimum and Maximum Values to define an inclusive data range for **blank wells.** The valid limits are reader-dependent.

   Explanation:

   During data reduction, the OD, RFU, or RLU value in each **blank** well is verified against this range. If a value falls outside the range, two options are available:

   - **Discard the out-of-range value.** If Discard Out-of-Range Values is selected, the blank well is reported as **OUT** and is excluded from the calculation of the blank mean.
   - **Retain the out-of-range value.** If Discard Out-of-Range Values is **not** selected, KCjunior automatically sets the raw OD, RFU, or RLU value to the Minimum or Maximum Blank Limits value, as appropriate. This new value is displayed inside brackets **[**0.010**]**, and is included in the calculation of the blank mean.

*Protocol database versions **1.2 and greater** support the Precision parameters.*

*Select **About KCjunior** from the **Help** menu to see the version of the currently selected Protocol Database.*

6. Set the **Displayed Decimal Places** value to 0, 1, 2, or 3. This value overrides the setting under **Setup|Configuration, Reporting Options.** Values such as optical density measurements, transformation results, and concentrations will be reported with the number of decimal places defined in this field, unless Scientific Notation is enabled.

7. (Optional) Check **Scientific Notation (Raw Data / Blanks)** to report Raw and Blanked data in scientific notation. Check **Scientific Notation (Transforms/Concentrations)** to report Transformation results and calculated Concentrations in scientific notation.

   If Scientific Notation is not enabled, KCjunior automatically displays a very small number like 0.00004 as 0.000 instead of as 4e-05.

Read Method Parameters

The configuration of the **Read Method** parameters controls plate reading. The availability of individual Read Method parameters depends on the current reader type, and can include read method type, wavelengths, filter sets, sensitivity, kinetic settings, number of plates, microplate geometry, incubation and shaking instructions, and more.

Before Defining Read Method Parameters

Because the availability of various Read Method parameters is reader-dependent, ensure that the desired **Reader Type** is selected under **Setup|Readers** and also in the **Current Reader** menu *before* defining these parameters. The reader does *not* need to be connected to the computer to define Read Method parameters.

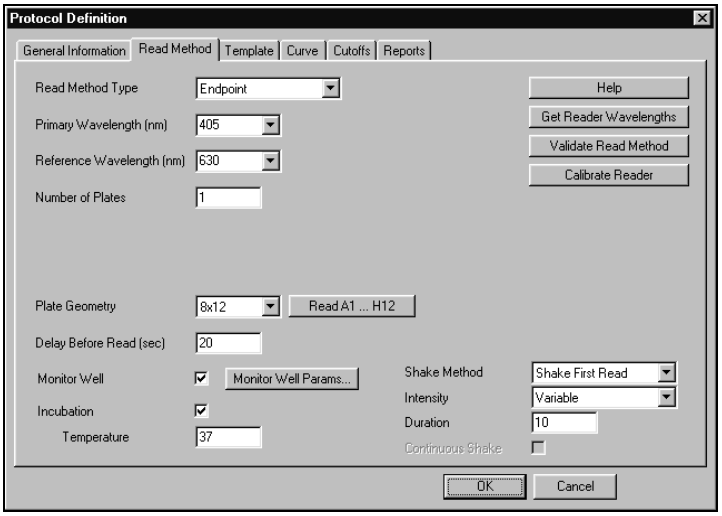
Selecting a Read Method Type

The **Endpoint** and **Kinetic** read methods are available for all readers supported by KCjunior. For certain readers (such as the PowerWave and the µQuant), the **MultiWavelength** and **Spectral Scan** read methods are available. For the FLx800 with its optional reagent dispenser, the **Dispense / Read** read method is available.

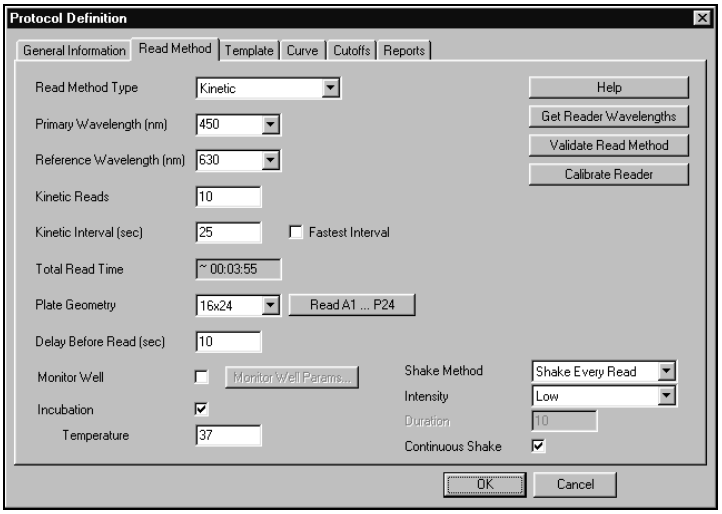
To select a **Read Method Type,** click to open the drop-down list and select Endpoint, Kinetic, MultiWavelength, Spectral Scan, or Dispense / Read.

- After a type is selected, related definable parameters automatically become accessible. For example, if *Kinetic* is chosen, data entry fields such as *Kinetic Reads* and *Kinetic Read Interval* becomes accessible.
- Sample Read Method dialogs are shown on the following pages.

Read Method Dialogs for Absorbance Readers

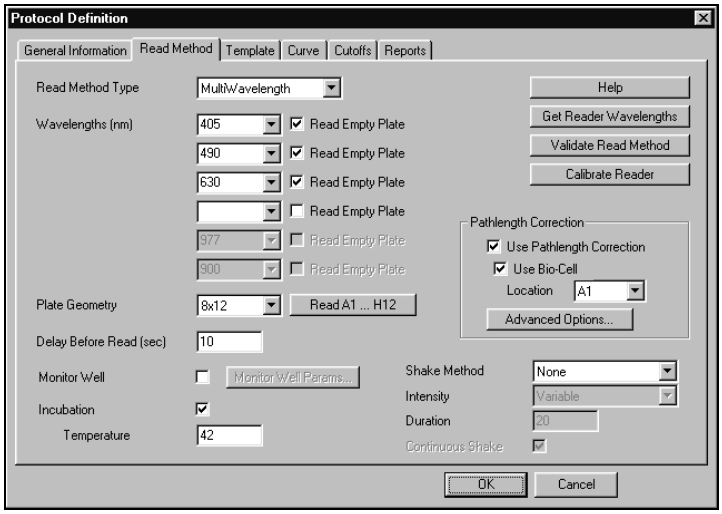


- During an **Endpoint Read,** every well on the plate is read at one or two wavelengths. One optical density (OD) value is reported for each well.

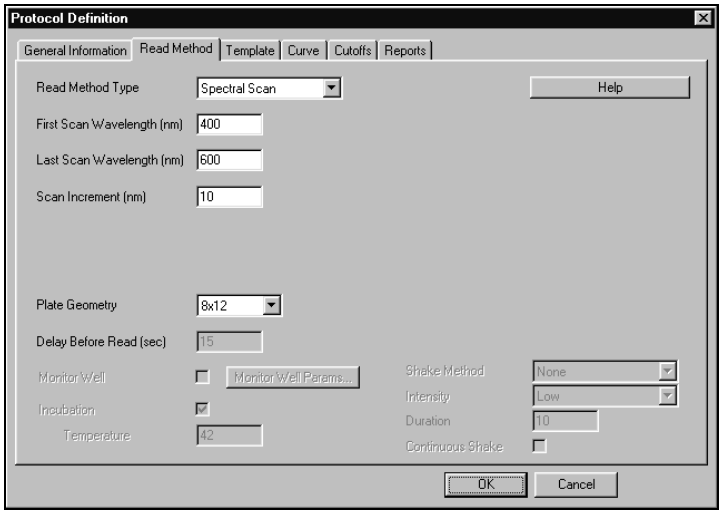


- During a **Kinetic Read,** every well on the plate is read a defined number of times at one or two wavelengths. One optical density value is reported for each well for each plate read. A graph showing OD vs. Time is reported for each well.

(Read Method Dialogs for Absorbance Readers)

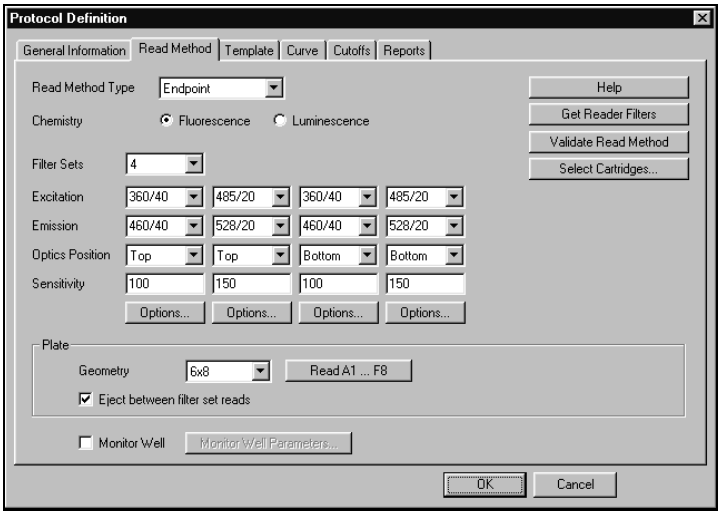


- During a **MultiWavelength Read,** the plate is read at each defined wavelength (up to 6). The optical density value measured at each wavelength is reported for each well. **Note:** The Pathlength Correction feature can be used with MultiWavelength reads.

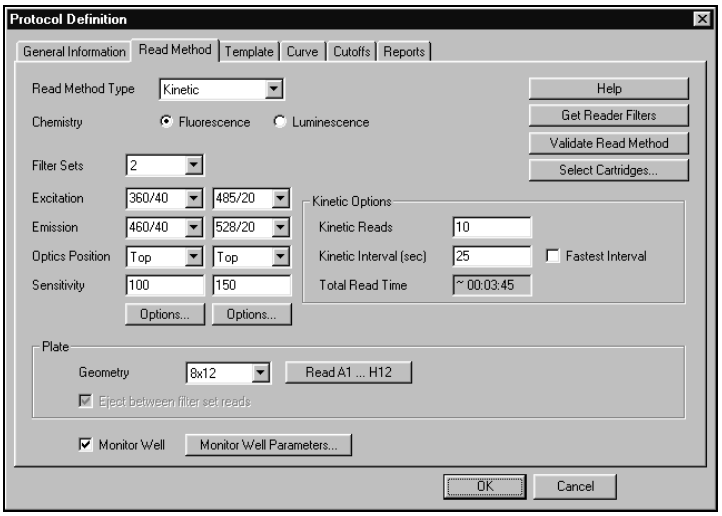


- During a **Spectral Scan,** multiple readings are taken across a wavelength range on up to 8 wells. A graph showing absorbance or transmission vs. wavelength is reported for each well.

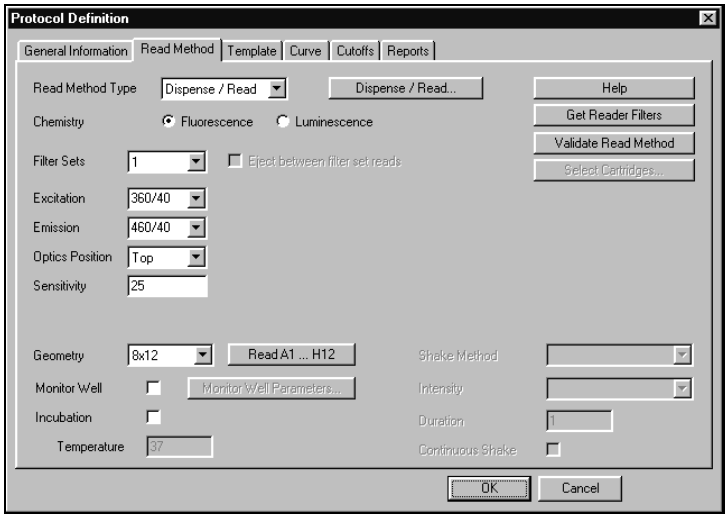
Read Method Dialogs for Fluorescence/Luminescence Readers



- During an **Endpoint Read,** the plate is read from one to six times, depending on the number of filter sets specified. One Relative Fluorescence Unit (RFU) or Relative Luminescence Unit (RLU) value is reported for each well *for each filter set.*



- During a **Kinetic Read,** the plate is read a defined number of times, using one or two filter sets. One Relative Fluorescence Unit (RFU) or Relative Luminescence Unit (RLU) is reported for each well, *for each kinetic read and for each filter set.*
  A graph showing RFU/RLU vs. Time is generated for each well.



- During a **Dispense / Read** sequence (FLx800), fluid is dispensed before or during sample measurement. KCjunior generates a kinetic curve showing Relative Fluorescence Units (RFU, Y axis) versus Time (X Axis) for each well.

Defining Read Method Parameters

After the Read Method Type is selected, the definable parameters automatically adjust accordingly.

All possible Read Method parameters are described on the following pages. See below for a list of the remaining topics in this section.

- The following topics are specific to **absorbance** readers:

  Selecting Primary and Reference Wavelengths, p. 5-16.

  Selecting Multiple Wavelengths, p. 5-16.

  Defining the Spectral Scan Wavelength Range, p. 5-20.

  Defining the Number of Plates to be Read, p. 5-20.

  Selecting a Read Mode, p. 5-35.

- The following topics are specific to **fluorescence/ luminescence** readers:

  Choosing the Chemistry, p. 5-21.

  Specifying Dispense / Read Options, p. 5-21.

  Selecting Filter Wheel Cartridges, p. 5-23.

  Defining Filter Sets, p. 5-24.

  Specifying Filter Set Options, p. 5-27.

  Ejecting the Plate Between Filter Sets, p. 5-30.

- The following topics apply to most readers:

  Defining Kinetic Parameters, p. 5-31.

  Selecting the Plate Geometry, p. 5-31.

  Setting a Time Delay Before Reading, p. 5-32.

  Monitoring Wells, p. 5-32.

  Defining Incubation Parameters, p. 5-34.

  Defining Shake Parameters, p. 5-34.

  Validating Read Method Parameters, p. 5-36.

  Retrieving Reader Wavelengths/Filters, p. 5-36.

  "Sending" Reader Wavelengths, p. 5-36.

  Calibrating the Reader, p. 5-37.

  Calibrate Before First Read, p. 5-37.

Selecting Primary and Reference Wavelengths

If a **Primary Wavelength** is specified alone, the plate is read at a single wavelength.

Dual wavelength readings significantly reduce optical interference caused by scratches or fingerprints on the microplate.

If a **Reference Wavelength** is also specified, the plate is read twice, once at each wavelength. During data reduction, KCjunior automatically calculates the **Delta OD** as the difference between the two readings.

To select **Primary** and **Reference Wavelengths:**

1. Select a Read Method Type of **Endpoint** or **Kinetic.**
2. Enter the **Primary** and **Reference Wavelengths** in the appropriate fields.

   Alternatively, click the **Get Reader Wavelengths** button to retrieve wavelength information from the reader. The reader must be connected to the computer, and communication parameters must be set properly.

   Following successful retrieval, use the drop-down menus to select the Primary and Reference Wavelengths.

Selecting Multiple Wavelengths

For certain reader models, KCjunior provides the ability to read a plate at multiple (up to six) wavelengths. Common reasons for reading a plate at multiple wavelengths include:

- Calculating the ratio of measurements at two different wavelengths.
- Determining a solution's peak absorbance by measuring at several wavelengths.

To select **Multiple Wavelengths:**

1. Select a Read Method Type of **MultiWavelength.**
2. Enter up to six wavelength values in the **Wavelengths** fields.

   Alternatively, click the **Get Reader Wavelengths** button to retrieve wavelength information from the reader. The reader must be connected to the computer, and communication parameters must be set properly.

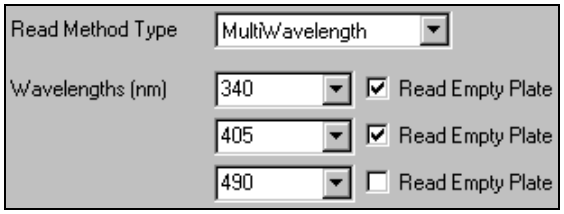
   Following successful retrieval, click the drop-down arrows to select the desired wavelengths.

Instruct KCjunior to calculate and report the difference in measurements between an empty plate and the sample plate.

3. (Optional) For any defined wavelength, check **Empty Plate** to perform two reads at the corresponding wavelength – the first read on an empty plate, and the second on the sample plate. KCjunior automatically subtracts the value of each well of the empty plate from the corresponding well of the sample plate.

For example, examine the **Wavelength** setup shown below:

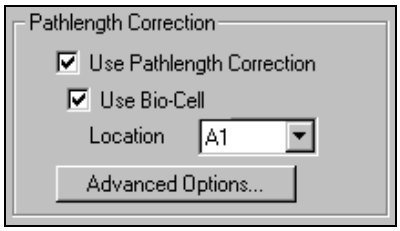
The "Empty Plate" can represent an empty dry plate or a plate filled with solution for blanking.



The empty plate will be read at 340 nm and 405 nm. The sample plate will be read at 340 nm, 405 nm, and 490 nm.

4. (Optional) Check **Use Pathlength Correction** to normalize absorbances to 1 cm. This option is only available for readers that support the MultiWavelength read method and wavelength values up to 999 nm.

Pathlength Correction *requires the 5th and 6th filters to be set either to* ***<None>*** *or to the* ***Test*** *and* ***Reference Wavelengths,*** *respectively.*



- If Pathlength Correction is enabled, KCjunior:
  (1) Calculates the **pathlength** for each well.
  (2) Calculates the **corrected absorbance** for each well.
  (3) Displays the corrected absorbance for each well under the **Pathlength Correction** results tab.
- KCjunior calculates each well's **pathlength** using the following formula:

  $(A_{977\ nm} - A_{900\ nm})well\ /\ (A_{977\ nm} - A_{900\ nm})1.0\ cm\ water$

  The well is measured at 977 nm and again at 900 nm, then the difference is calculated. The result is divided by the absorbance of water at 1 cm.

  977 nm and 900 nm represent default **Test** and **Reference Wavelengths,** respectively. These wavelengths can be changed under **Advanced Options.**

  KCjunior uses a default value of **0.18** for the absorbance of water at 1 cm. This default value can be overridden by changing the Constant Value under Advanced Options.

  Alternatively, click **Use Bio-Cell** to calculate the absorbance of water at 1 cm value using Bio-Tek's Bio-Cell™ plate. Set the **Location** of the cuvette in the plate.

The pathlengths can be included on the ***Matrix Report.*** *See "Selecting Options for the Matrix Report" at the end of this chapter.*

- KCjunior calculates the **corrected absorbance** for each well according to the following formula:

  $A_{wavelength}$well ÷ pathlength of well

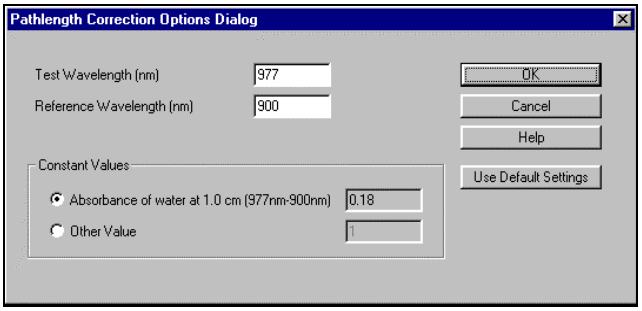
  The measurement taken at the user-defined wavelength is divided by the calculated pathlength.

  The **Raw Data** value of the well is used in the correction formula, unless an empty plate is read or blank subtraction is performed.

  If an empty plate is read, the difference (or **Result**) in the Raw Data readings between the full and empty plate is used in the formula.

  If blank subtraction is performed on the Raw Data or the Result value, the **Blanked Data** value is used in the formula.

- Click the **Advanced Options** button to change the Test or Reference Wavelength values, or to change the Constant Value.



  The valid range for the **Test** and **Reference Wavelengths** fields is 200-999 nm.

  To define a Constant Value other than 0.18 for use with calculating pathlengths, click **Other Value,** then enter the desired number.

  Click **Use Default Settings** to reset the Test and Reference Wavelengths to 977 and 900 nm, respectively, to reset Other Value to 1, and to reselect the use of the Absorbance of water at 1 cm value.

Example 1:

A protocol specifies the following:

- MultiWavelength read method type
- Microplate read at 405 nm
- Pre-read an empty plate at 405 nm
- Pathlength Correction is enabled
- The default value of **0.18** represents the absorbance of water at 1 cm (977-900 nm).

  The **empty** plate is read at 405 nm, to obtain the blanking absorbances for each well.

  The **sample** plate is initially read at the Test and Reference Wavelengths. Well A1 readings at 977 and 900 nm are 0.175 and 0.025, respectively. The **pathlength** for A1 is calculated as (0.175-0.025)/0.18, or **0.8333.**

  The sample plate is then read at 405 nm, and the empty plate values are subtracted to determine the **405 Result** values. The 405 Result value for well A1 is 2.576. The **corrected** value equals 2.576 / 0.8333, or **3.091.**

Example 2:

A protocol specifies the following:

- MultiWavelength read method type
- Microplate read at 405 nm
- Do not pre-read an empty plate
- Pathlength Correction and Use Bio-Cell are enabled
- Blank wells are defined in the well ID template

  The **Bio-Cell** plate is read at the Test and Reference Wavelengths. The absorbance of water at 1 cm (977-900 nm) value is **0.182.**

  The **sample** plate is initially read at the Test and Reference Wavelengths. Well H1 readings at 977 and 900 nm are 0.208 and 0.034, respectively. The **pathlength** for H1 is calculated as (0.208-0.034)/0.182, or **0.956.**

  The sample plate is then read at 405 nm, and after blank well subtraction, the **405 Result** value for well H1 is 2.619. The **corrected** value equals 2.619 / 0.956, or **2.740.**

Defining the Spectral Scan Wavelength Range

During a Spectral Scan, the reader takes multiple measurements across a wavelength range on each of up to 8 wells. A graph showing absorbance or transmission vs. wavelength is reported for each well.

To define the **Spectral Scan wavelength range:**

1. Select the **Spectral Scan** Read Method Type.
2. Enter the **First Scan Wavelength** and **Last Scan Wavelength.**

   The valid ranges for the **First** and **Last Scan Wavelength** fields are 200-998 nm and 201-999 nm, respectively. The First setting must be lower than the Last setting.
3. Set the **Scan Increment** to determine which wavelengths will be used for measurement within the wavelength range.

   For example, if the wavelength range is 200-600 nm with a **Scan Increment** of 10 nm, measurements are taken at 200, 210, 220, … 600 nm.

Defining the Number of Plates to be Read

Tip: *For a quantitative endpoint assay that calculates results based on a stored curve, use the* ***Number of Plates*** *feature to read up to 100 plates.*

Up to 100 successive plate reads can be performed with one **Endpoint** protocol. The plate carrier is automatically ejected after each read, to allow for submittal of a different plate.

To define the **Number of Plates** to be read:

1. Enter the number of plates to be read in the **Number of Plates** field.
   - At run-time, KCjunior provides the option to assign a **Plate ID** to each individual plate.
   - If the attached reader is equipped with a plate barcode scanner and **Use reader's barcode scanner for plate ID** is selected under Setup|Configuration, General, KCjunior scans the plate before each read to capture the plate ID.
   - The raw data for all of the endpoint reads are stored as one record, under one **Results ID,** in the Results Database.

Note: The following sections discuss read method parameters that are specific to **fluorescence** and **luminescence readers.** Skip ahead to page 5-31 for information on defining kinetic parameters, enabling shaking or incubation, validating read method parameters, and more.

For general information on Read Method parameters, or for instructions on selecting a Read Method type, see page 5-10.

Choosing the Chemistry

Click the radio button associated with the desired chemistry, **Fluorescence** or **Luminescence.**

- KCjunior sets certain parameters in accordance with the chosen chemistry. For example:
  - Measurements are reported as either **RFU** or **RLU,** to represent Relative *Fluorescence* Units or Relative *Luminescence* Units, respectively.
  - A warning message appears if the Excitation filter is not set to "Plug" in a Luminescence protocol (a plug is recommended for Luminescence readings).
- For Luminescence protocols, the instrument's **lamp** automatically turns off just prior to plate reading. Refer to the instrument operator's manual for more information on the lamp.

Specifying Dispense / Read Options

If the current reader is the FLx800 and it is equipped with the reagent dispensing module, you can choose to dispense reagent before or during sample measurement.

To define **Dispense / Read** options:

1. Ensure that the **Read Method Type** is set to Dispense / Read.
2. Click the **Dispense / Read** button.
3. If a read will be performed, check **Perform Read.** If no read will be performed, skip to step 8.
4. If performing a read, choose **Well Mode** or **Plate Mode.**
   - In Well Mode the FLx800 reads each well individually for a specified number of times using 1 or 2 sampling windows, dispensing reagent as specified in Dispense Parameters (see step 8). This mode is typically used for fast kinetic reactions.
   - In Plate Mode the FLx800 reads a whole or partial plate for a specified number of times, dispensing reagent as specified in Dispense Parameters (see step 8).

5. If reading in Well Mode, select the number of **Sampling Windows,** 1 or 2.
6. For one or both Sampling Windows:
   - Enter a **Lag Time** from 0 to 600 seconds. This is the delay before the first Sample is read.

     Note: For two Sampling windows, KCjunior automatically calculates the Sampling Start time for the second window. The second window begins immediately after the first finishes, unless a Lag Time greater than 0 is specified for the second window.
   - Enter the total number of **Samples** (measurements to be taken) per well, from 1 to 300.
   - Enter the **Sample Interval** (the delay between sample measurements), from 0.02 to 12 seconds.
   - (Optional) Check **Light Shutter** to block the light when not measuring to inhibit photo bleaching.
7. If reading in Plate Mode:
   - Enter the total number of **Kinetic Reads,** from 1 to 300.
   - Enter a **Kinetic Interval** (delay between reads) of 1 to 9999 seconds.
   - (Optional) Check **Light Shutter** to block the light when not measuring to inhibit photo bleaching.
8. If performing a dispense, check **Perform Dispense.**
9. (Optional) If no read will be performed, or if a Well Mode read will be performed, choose a **Tip Prime Option** of **Before First Well** or **Before Every Well** and then enter a dispense tube **Prime Volume** of 5 to 20 ul.
   - **Tip:** If there is a long duration between dispenses from well to well, consider priming Before Every Well to compensate for any fluid loss at the dispense tip due to evaporation.
10. (Optional) If a Plate Mode read will be performed, check **Prime Tip Before Start of Processing** and then enter a dispense tube **Prime Volume** from 5 to 20 ul.
11. Enter a reagent **Dispense Volume** from 5 to 1000 ul.
    - The *minimum* Dispense Volume varies depending on the Dispense Rate selected.
12. Select a fluid **Dispense Rate,** of 1, 2, 3, or 4.
    - With each rate selection, the corresponding ul/sec value is displayed.

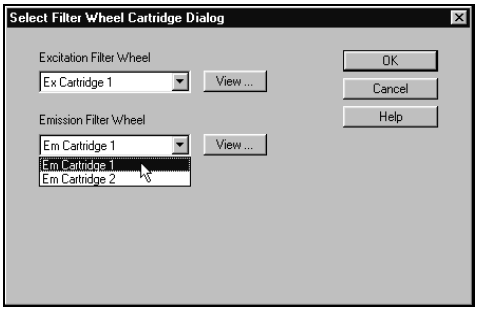
13. (Optional) If no read will be performed, or if a Well Mode read will be performed, enter a **Lag Time** from 0 to 6000 seconds. This is the delay before the dispense.
    - **Tip:** If two Sampling Windows are defined and you wish to dispense between the two windows, enter a Lag Time that matches the calculated Sampling Start time.
14. If a Plate Mode read will be performed, enter an **Inject on Interval** value from 1 to the number of Kinetic Reads. This is the read number at which the dispense will occur.
    - **Note:** The kinetic curve will display a green line on the X axis (Time) to indicate when the dispense occurred.
15. Click **OK** to save the changes and return to the Read Method dialog.
    - **Important:** If tip priming will be performed, make sure that the **tip priming trough** is installed in the instrument (refer to the operator's manual for placement instructions).

Selecting Filter Wheel Cartridges

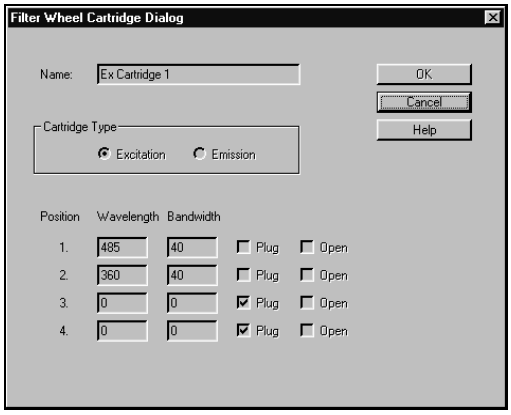
For a FLx800 that is used for a variety of applications, multiple Excitation and/or Emission filter wheel cartridges may be prepared and installed as needed. The contents of Excitation and Emission filter wheel cartridges are defined under Setup|Configuration. You can then select the cartridges to be associated with each protocol.

To select filter wheel cartridges:

1. Ensure that under **Setup|Configuration|Filter Wheel Cartridges,** Excitation and Emission cartridges are defined.
2. Click the **Select Cartridges** button from the Read Method dialog. The Select Filter Wheel Cartridge dialog appears.



3. Using the drop-down menus, **select** the desired Excitation and Emission Filter Wheel cartridges.
4. After selecting a cartridge, clicking **View** displays the cartridge's contents.



- Click **Cancel** to return to the Select Filter Wheel Cartridge dialog.

5. Click **OK** to save the changes and return to the Read Method dialog. The contents of the Excitation and Emission drop-down selection boxes now match the selected cartridges.

Defining Filter Sets

Each **Filter Set** specifies the Excitation filter, Emission filter, optics position, sensitivity setting, and sampling information to be applied during one plate read.

- One to six filter sets can be defined for an **Endpoint** protocol.
- One or two filter sets can be defined for **Kinetic** and **Dispense / Read** protocols.
  - **Note:** Dispense / Read protocols have special filter position requirements. See "Filter Wheel Cartridges" in *Setting Up the System* for more information.

There are many applications for the use of **multiple filter sets,** including:

- Comparison of results among various sensitivity settings with otherwise identical filter settings.
- Assays that have more than one fluorophore per well.
- Comparison of results between the top and bottom optics positions (if the reader is equipped with dual-optics). Select two filter sets with identical settings, one for the top probe and the other for the bottom probe.
- Comparison of results among various sampling settings.

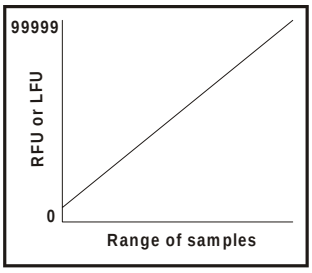
To define **Filter Sets:**

1. Ensure that the **Read Method Type** and **Chemistry** settings are correct.

2. If the reader is connected to the computer and turned on, click **Get Reader Filters** to update KCjunior's internal filter table with the reader's current settings.
3. Select the number of **Filter Sets** (1 to 6 for endpoint, 1 or 2 for kinetic).
4. Perform steps 5 through 8 **for each filter set.**
5. Select an **Excitation filter** and an **Emission filter.**
   - The **Excitation filter** selects a narrow band of light to which the sample will be exposed. The **Emission filter** selects a band of emitted light to be measured by the instrument's photomultiplier.
   - Valid selections include a wavelength/bandwidth combination (in nanometers), "Plug" to indicate the presence of a plug, and "Open" to indicate the absence of a filter or plug.
   - If the desired filter cannot be found within the drop-down list, manually enter the wavelength and bandwidth values.

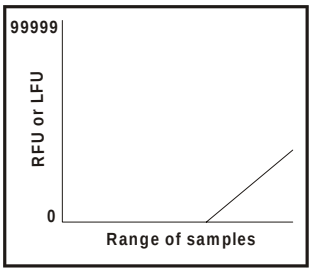
     The correct format is `wavelength/bandwidth`, in nanometers. For example, **485/20** represents the 485 nm wavelength with a bandwidth of 20 nm. This combination permits the transmission of light from 475 to 495 nm.

     Each filter is imprinted with its wavelength/bandwidth combination.
   - To prevent damage to the photomultiplier, KCjunior (1) prohibits the saving of a protocol with overlapping Excitation and Emission wavelengths defined; or (2) provides a warning if the wavelengths are considered to be "too close", but does not prohibit the saving of the protocol.
   - Just prior to a plate read, KCjunior communicates with the reader to see if its filter table contains all of the filter/bandwidth combinations defined in the protocol.
6. Choose an **Optics Position** of Top or Bottom.
   - Not all fluorescence/luminescence readers are equipped with two probes. When a plate read is initiated, KCjunior communicates with the reader to see if the selected Optics Position is supported.
   - If the reader is connected and is turned on, click the **Validate Read Method** button to see if the currently selected Optics Position is supported.



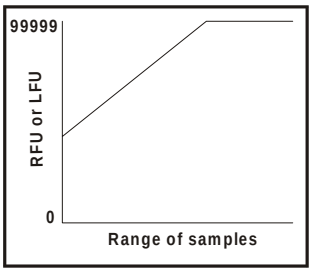
"Optimum" sensitivity

Samples fall within the 0 to 99999 RFU/RLU range. Lowest values are above 0.



Sensitivity is too low

Some samples are reported as 0 RFU/RLU.



Sensitivity is too high

Some samples are reported as 99999 RFU/RLU.

7. Specify the **Sensitivity** setting of the photomultiplier, unless automatic sensitivity adjustment will be used (see next step).

   The signal from the range of fluorescence and luminescence assays can be very weak, very strong, or anywhere in between. The photomultiplier (PMT) is the device inside a fluorometer that measures the light emitted from the sample.

   For each assay the **sensitivity** (gain) of the PMT should be adjusted to ensure that the signals from all wells fall within the appropriate dynamic range of 0 to 99,999 relative fluorescence or luminescence units (RFU or RLU).

   The drawing on the left compares results with an *optimum* sensitivity setting with settings that are *too low* or *too high.*

   Tips for selecting the "optimum" sensitivity:

   - Assays using **96-** (or fewer) **well** plates typically require sensitivities between 75 and 190. Assays using **384-well** or other higher-density plates will likely require higher sensitivities because of the narrower optical probes.
   - One method for determining the **optimum sensitivity** for an assay is to define multiple filter sets with the same parameters, varying only the sensitivity setting. Another method is to enable KCjunior's Automatic Sensitivity Adjustment feature (see "Specifying Filter Options").
   - As the sensitivity setting increases, the measurement values increase. If many wells result in **OVERFLOW** (indicating RFU or RLU values greater than 99,999), the sensitivity setting is probably too high.
   - If each well contains **more than one fluorophore,** one of which might give brighter or weaker results, assign each filter set its own sensitivity.
   - If the Sensitivity field is **dimmed and inaccessible,** the Automatic Sensitivity Adjustment feature is enabled.

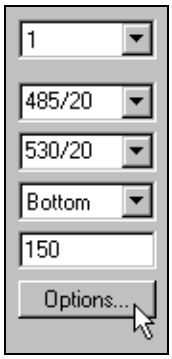
8. Click the **Options** button to specify **Sampling** parameters and/or to enable the **Automatic Sensitivity Adjustment** feature.

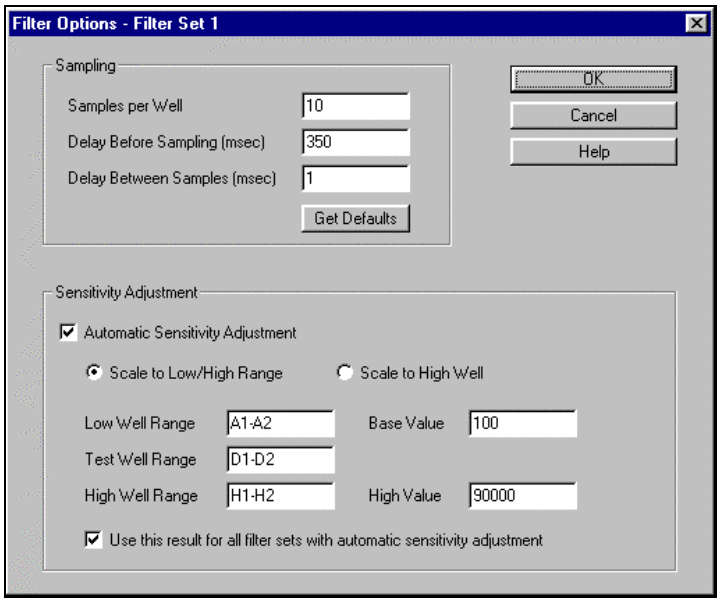
   See "Specifying Filter Set Options" for further information.

Specifying Filter Set Options

Each filter set has its own configuration of options for well sampling, and for automatic sensitivity adjustment (if required).

Click the Options button to define Sampling and/or Automatic Sensitivity options for a filter set.





Sampling

KCjunior provides three **Sampling** options to specify the well reading characteristics for a filter set.

Note: Click the **Get Defaults** button to set all three options to their original default settings.

To define the Sampling options:

1. Specify the **Number of Samples** (measurements) to take per well. The valid entry range is 1 to 255 measurements. The value reported for each well represents the average of its measurements.
   - The reader spends approximately 10 milliseconds performing *each* measurement.
   - Usually, the more samples per well the better the CV's, although selecting more than 10 samples per well typically results in only marginal improvement. 10 is the recommended combination of sensitivity and speed.

2. Set the **Delay Before Sampling,** in milliseconds. This is the pause time to allow plate contents to settle before the measurements begin. The valid entry range is 10 to 2550 milliseconds, in 10 ms increments.
   - 350 milliseconds is the recommended setting to ensure the plate is definitely stopped for measurement and for the minimum amount of time.
3. Set the **Delay Between Samples,** in milliseconds. This is the time delay between samples. The valid range is 0 to 255 milliseconds.
   - For fluorescence assays, a delay of 10 milliseconds is recommended. **Note:** Delays longer than 200 milliseconds may "bleach" the samples.
   - For luminescence assays, longer delays between samples may result in better CV's.

Automatic Sensitivity Adjustment

The Help system shows how to define pre-reading parameters for a sample assay:

1. *Click the Help button from within the Filter Options dialog.*
2. *Click on the last option, "Examples…".*

KCjunior provides an **Automatic Sensitivity Adjustment** feature as an alternative to the more manual method of defining multiple filter sets with varying sensitivity settings.

If this feature is enabled, KCjunior instructs the reader to perform several pre-readings on the microplate. In general, these readings are performed on user-specified wells using incremental sensitivity settings. Each increase in sensitivity raises the RFU/RLU values of all of the wells. Certain wells are monitored to ensure that they, and hence the assay, do not overrange. KCjunior reviews the results of the pre-readings and selects the "optimum sensitivity" for plate reading.

KCjunior provides two options for Automatic Sensitivity Adjustment:

- **Scale to Low/High Range.** If this feature is enabled, the instrument performs several pre-readings of the specified **Low, Test,** and **High Wells.** With each reading, the sensitivity (gain) of the photomultiplier is increased. The target wells are monitored to ensure that they, and hence the assay, do not exceed the defined **High Value.**

  KCjunior reviews the results of the pre-readings and selects the "optimum sensitivity" – the one that gives the best signal-to-noise ratio when comparing one (higher) set of wells with another (lower) set of wells. The entire microplate is then read using this sensitivity setting.

- **Scale to High Well.** If this feature is enabled, the instrument performs several pre-readings of the specified **High Well.** With each reading, the sensitivity (gain) of the photomultiplier is increased. The target well is monitored to ensure that it, and hence the assay, does not exceed the defined **High Value.**

  Measurements stop when the target well reaches the High Value. The sensitivity that produced the RFU/RLU closest to the High Value (without exceeding it), is chosen for the microplate read.

To enable Automatic Sensitivity Adjustment and define the pre-reading parameters for the **Scale to Low/High Range** option:

Note: For any of the following parameters, enter a single well location (ex. A1), or a "rectangle" of well locations (ex. B1-C2).

1. Specify the **Low Well Range,** to define the well(s) representing the expected Base Value (see step 4).
   - Low Wells are usually blank wells, buffer wells, zero standards, or negative controls, and are expected to yield a low RFU or RLU.
   - This range must not overlap the Test Well Range.
2. Specify the **Test Well Range,** to define the well(s) to be used in conjunction with the Low Wells to determine signal-to-noise ratios.
   - Test Wells are usually low standards or low positive controls, and are expected to yield a mid-range RFU or RLU.
   - For best results, designate as the Test Wells those wells on the plate generating a signal at least 50% greater than the Low Wells.
   - This range must not overlap the Low Well Range.
3. Specify the **High Well Range,** to define the well(s) representing the expected High Value (see step 5).
   - High Wells are usually the highest standards or high positive controls, and are expected to yield a high (but not overrange) RFU or RLU.
   - This range must not overlap the Low Well Range. It can, however, overlap the Test Well Range.

4. Specify the **Base Value.** This is the fluorescence or luminescence value that the Low and Test Wells must exceed during sensitivity determination, to ensure that the plate produces values that are large enough to be meaningful.
   - The default (and recommended) setting is 100 RFU/RLU.
5. Specify the **High Value.** This is the fluorescence or luminescence value that the High Well(s) should approach but not exceed, in order to produce a valid signal-to-noise ratio calculation.
   - The default setting is 90,000 RFU/RLU, and the maximum value is 99,999. Practically, this value could be anywhere from 10,000 to 90,000.
6. If this is Filter Set #1, checking **Use Result for All Filter Sets** instructs KCjunior to use the sensitivity determined here for all other filter sets with Automatic Sensitivity Adjustment enabled.

To enable Automatic Sensitivity Adjustment and define the pre-reading parameters for the **Scale to High Well** option:

1. Specify the **High Well.** This is the target well to be measured.
   - The High Well usually represents a high standard or high positive control, and is expected to yield a high (but not overrange) RFU or RLU.
2. Specify the **High Value.** This is the fluorescence or luminescence value that the High Wells should approach but not exceed.
   - The default setting is 90,000 RFU/RLU, and the maximum value is 99,999. Practically, this value could be anywhere from 10,000 to 90,000.
3. If this is Filter Set #1, checking **Use Result for All Filter Sets** instructs KCjunior to use the sensitivity determined here for all other filter sets with Automatic Sensitivity Adjustment enabled.

Ejecting the Plate Between Filter Sets

For fluorescence/luminescence protocols, enable **Eject between filter set reads** to:

- Provide the ability to add reagent to the same plate between readings.
- Enable the reading of an initial blank plate followed by the sample plate.
- Enable the reading of up to 6 different plates using one protocol

Defining Kinetic Parameters

To define **Kinetic** parameters:

1. Select the **Kinetic** Read Method Type.
2. Enter the number of **Kinetic Reads.** The Kinetic Read value represents the number of times the entire plate will be read.
3. Enter the **Kinetic Interval** in seconds. The Kinetic Interval is the time delay between Kinetic Reads.

Let KCjunior automatically calculate the minimum Kinetic Interval.

- Alternatively, allow KCjunior to select the minimum kinetic interval. Check the **Fastest Interval** box to instruct KCjunior to calculate the minimum kinetic interval appropriate for the currently selected reader type. This helps to ensure the fastest plate read possible.
- After the Kinetic Reads and Kinetic Interval have been entered, KCjunior automatically calculates the approximate **Total Read Time** and displays it in the ~HH:MM:SS format.

Note: *If shaking is enabled, the Shake Duration is included in the calculation of the Total Read Time.*

4. For the ELx800, PowerWave, PowerWaveX, µQuant, and FLx800 readers:

*If the reader is set to run in **Rapid Mode,** the minimum kinetic interval can be set to a lower value than the theoretical minimum value calculated by KCjunior. Refer to the reader's user manual for instructions on changing the reading mode.*

- When Fastest Interval is selected, KCjunior calculates a *theoretical* minimum kinetic interval.
- If the reader is connected to the computer, click the **Validate Read Method** button to verify this value. If the KCjunior theoretical value is too low, the reader returns a valid range for the **Kinetic Interval** field.
- If the reader is not currently connected to the computer and **Fastest Interval** is selected, the Kinetic Interval is verified just prior to a plate read. If the value is too low, the reader returns a valid range for the **Kinetic Interval** field.

Selecting the Plate Geometry

The reader does not need to be connected to the computer to select a Plate Geometry.

The **Plate Geometry** represents the number of rows and columns in a microplate. For example, a **Plate Geometry** of 8x12 represents the standard 96-well plate.

8x12 is the default for every reader type. Depending on the current reader, additional geometry selections can include 2x3, 3x4, 4x6, 6x8, 6x10(Terasaki), 6x12(Terasaki), 8x12 (Hellma), 8x12 (Metric), 8x12(Terasaki), 16x24, and BioCell.

To select a **Plate Geometry:**

1. Ensure that under **Setup|Reader,** the desired **Reader Type** is currently selected.
2. Use the drop-down menu to view a list of available geometry options for the current reader type.
3. Click once on the desired **Plate Geometry** type.
4. (Optional) If **partial plate reading** is supported by the current reader, click the button to the right of the Geometry field to define the well(s) to be read.
   - Define a "rectangle" of wells to be read by entering the **First Well** and **Last Well,** or by **highlighting** the desired wells in the grid.

     For example, to read columns 4 through 7 of a 6x8 plate, set First Well to A4 and Last Well to F7, or highlight columns 4, 5, 6, and 7 in the grid.
   - To set the First and Last Well values to coordinate with the currently selected Geometry, click **Read Full Plate.**
   - Click **OK** to return to the Read Method dialog.

Setting a Time Delay Before Reading

Set a Delay Before Read to allow time for stabilization.

Use this feature to set a delay between the time the operator initiates the plate read and the time the plate read actually begins.

Notes: For MultiWavelength reads, if Read Empty Plate is enabled, the delay begins after the empty plate(s) are read. For multiple-plate Endpoint reads, the delay occurs before *each* plate is read.

To set a **Delay Before Read:**

1. Enter the time (in seconds), in the **Delay Before Reading** field.

Monitoring Wells

If the currently selected reader supports well monitoring, the **Monitor Well** option is available.

This feature enables the instrument to repeatedly read a single well or a group of wells and evaluate the measurements against pre-defined criteria. Once the criteria are met, the plate read begins.

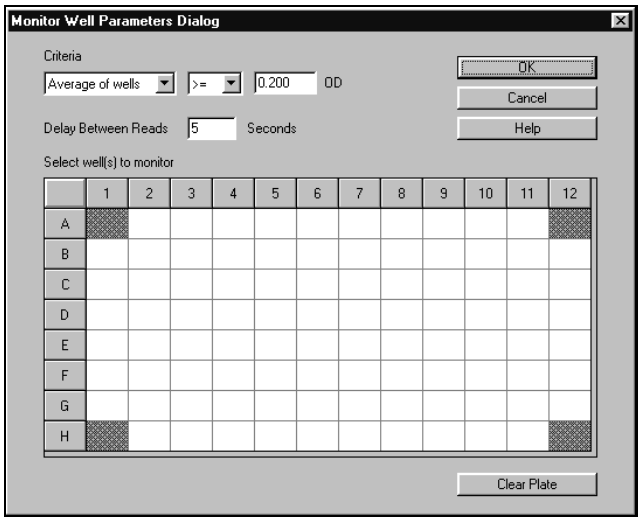
To define Monitor Well parameters:

1. Check the **Monitor Well** box.

2. Click the **Monitor Well Parameters** button. The Monitor Well Parameters dialog appears:

*KCjunior database versions **1.4** and greater support the Monitor Well feature.*

*From the main menu, select **Help**|**About KCjunior** to see the version numbers for the currently selected protocol and results databases.*



3. Specify the monitoring **Criteria:**

   - **Note:** For the EL311/312/340 readers, the criteria is limited to the entry of a **Comparison Value (OD).** When the selected well reaches the defined comparison value, monitoring stops and the plate read begins.
   - From the first drop-down list, select a statistic of **At Least One Well, Average of wells,** or **All Wells.**

     Note: If only one well will be monitored, select any statistic. The behavior will be the same for all three options.
   - From the second drop-down list, select a logical operator, **<, <=, >,** or **>=.**
   - Enter a **limit value.** This is the target measurement value for the selected well(s). The valid range is reader-dependent.
   - Examples:

     At Least One Well >= 0.050 OD: The plate read will begin when at least one of the selected wells is greater than or equal to 0.050 OD.

     Average of Wells > 0.300 OD: The plate read will begin when the average value of the selected wells is greater than 0.300 OD.

     All Wells < 80000 LFU: The plate read will begin when all of the selected wells measure less than 80000 LFU.

4. (Optional) Enter a **Delay Between Reads,** in seconds. This is the delay between readings of the selected well(s).

   - **0** indicates no delay.

5. Highlight **up to 8 wells** to be monitored.
   - If a well is **shaded,** it has been selected and will be monitored.
   - Any well(s) on the plate can be monitored, even if the protocol specifies only a partial plate read.
   - Click the **Clear Plate** button to clear the currently selected wells.
   - **Note:** For the EL311/312/340 readers, select only one well.
6. Click **OK** to save the settings and return to the Read Method parameters dialog.

Defining Incubation Parameters

If the currently selected reader supports plate incubation, the **Incubation** option is available.

To define **Incubation** parameters:

1. Ensure that under **Setup|Reader,** the desired **Reader Type** is currently selected.
   - The reader does not need to be connected to the computer to define Incubation parameters.
2. Check the **Incubation** box. The **Temperature** field becomes accessible.
3. Enter the setpoint temperature (°C) in the **Temperature** field. This is the temperature to which the plate chamber will be heated prior to plate reading. The valid range is from 20°C (or 4°C above room temperature, whichever is greater), to 50°C.

Defining Shake Parameters

If the currently selected reader supports plate shaking, the **Shake** parameters are accessible in the **Read Method** dialog.

To define **Shake** parameters:

1. Ensure that under **Setup|Reader,** the desired **Reader Type** is currently selected.
   - The reader does not need to be connected to the computer to define **Shake** parameters.
2. Select a **Shake Method** from the drop-down menu. Available options include None, Shake First Read and Shake Every Read.
   - If **Shake First Read** is selected, the plate is shaken prior to the first plate reading.
   - If **Shake Every Read** is selected, the plate is shaken prior to every plate reading. This option typically applies to the kinetic read method.

*Not every reader that supports plate shaking supports the **Variable** intensity setting.*

3. Select the **Shake Intensity.** Available options include Low, Medium, High, and Variable. If **Variable** is selected, the plate is shaken at varying intensities over the defined duration.
4. Set the **Shake Duration.** The valid range is 1-60 seconds.
5. (Optional) For kinetic protocols, if Shake Every Read is enabled, the **Continuous Shake** option is available. If selected, the plate shakes continuously between the kinetic reads.

Selecting a Read Mode

If the currently selected reader supports multiple **Read Modes,** choose a mode of Normal, Rapid, or Sweep:

- **Normal** mode is the default and its exact behavior is reader-dependent. In general this mode results in slower plate reading and yields the most precise results.
- With **Rapid** mode, reading is faster than with Normal mode.
- With **Sweep** mode, reading is very fast because the carrier "sweeps" the plate through the reader without stopping.

Notes:

The selection of one read mode over another generally depends on the desired outcome of the assay (better precision versus faster plate reading).

All readers that support multiple read modes support the Normal mode. Many readers also support the Rapid mode. The PowerWave Robotic instrument supports the Sweep mode (more readers may support this mode in the future).

Just before the plate read, KCjunior checks to see if the attached reader supports the Read Mode currently selected. If the reader does not support the current Read Mode, a different one must be selected.

Validating Read Method Parameters

The **Validate Read Method** button is available in the Read Method dialog for some readers. When this button is clicked, KCjunior communicates with the reader to verify the defined Kinetic Interval, and also verifies that all required fields contain valid entries.

To **validate** the read method parameters:

1. Ensure that under **Setup|Reader,** the desired **Reader Type** is currently selected, and that the communication parameters are set correctly.
2. Ensure the reader is attached to the computer and turned on.
3. Define the read method parameters.
4. Click the **Validate Read Method** button.

KCjunior attempts to communicate with the reader. If communication is successful, the parameters are validated and the results are displayed. If a change is required, KCjunior provides the necessary instructions.

Retrieving Reader Wavelengths/Filters

KCjunior provides the capability to retrieve wavelength/filter information from the attached reader, and store that information in the drop-down Wavelength or Filter selection menus.

To retrieve **Wavelength/Filter** information from a reader:

1. Attach the reader to the computer and turn it on.
2. Ensure that under **Setup|Reader,** the desired **Reader Type** is currently selected, and that the communication parameters are set correctly.
3. Click the **Get Reader Wavelengths** button (or the **Get Reader Filters** button) from the Read Method dialog.

KCjunior attempts to communicate with the reader. If communication is successful, the Wavelength/Filter drop-down menus are updated to match the reader's current settings.

"Sending" Reader Wavelengths

For the PowerWave, PowerWaveX, and µQuant readers: Just prior to plate reading, if the protocol contains wavelengths that are not present in the reader's filter table KCjunior instructs the reader to calibrate the new wavelengths.

Calibrating the Reader

The **Calibrate Reader** button is available in the Read Method dialog for readers that support calibration. When this button is clicked, the reader runs a calibration routine and performs a system test.

Note: For Spectral Scan reads, there is a **Calibrate Before First Read** checkbox instead of a Calibrate Reader button. See the next topic.

To **calibrate** the reader and perform a **system test:**

1. Ensure that under **Setup|Reader,** the desired **Reader Type** is currently selected, and that the communication parameters are set correctly.
2. Ensure the reader is attached to the computer and turned on.
3. Ensure that the **Wavelengths** are specified appropriately.

4. Click the **Calibrate Reader** button from the Read Method dialog.

   KCjunior attempts to communicate with the reader. If communication is successful, the reader performs calibration and then runs the system test.
5. When the system test is complete, the results are displayed. Click **OK** to close the results and return to the Read Method dialog.

Calibrate Before First Read

For Spectral Scan reads, the **Calibrate Before First Read** option is available.

- If this box is checked, the reader calibrates at all of the wavelengths defined in the protocol, just prior to plate scanning.
- If this box is not checked, the reader calibrates at only those wavelengths that have not yet been calibrated since the reader was turned on.

Templates

Within each KCjunior protocol, four unique **Templates** can be designed to indicate location and related information for **Well IDs, Dilutions, Transformations,** and **Sample IDs:**

- **Well Identifiers** play an important role in calculations such as blank subtraction, curve generation, and cutoff evaluation.
- **Dilutions** can be specified to indicate a reduction in the amount of concentration in solutions.
- **Transformations** are user-definable formulas that can be applied to one or more wells.
- **Sample IDs** can be defined to distinctly identify sample wells.

To design templates:

1. Choose to create or modify a protocol.
2. Click the **Template** tab. The **Well ID Template** appears.
3. Define or modify Well ID information, or click the **Dilutions, Transformations,** or **Sample IDs** radio button to select a different template type.

   After a template type is selected, related definable parameters automatically become accessible. For example, if the *Well ID Template* is selected, data entry fields such as *Well Type Selection*, *Blank Method*, and *[Replicate] Number* become accessible.

   Regardless of the template type, the information entry screens are conveniently arranged with rows and columns that align with the selected Plate Geometry.

   A sample **Template** information entry screen for the 8x12 plate geometry is shown below:

	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	BLK	SMP03	SMP03	SMP11	SMP11	SMP19	SMP19	SMP27	SMP27	SMP35	SMP35
B	STD01	STD01	SMP04	SMP04	SMP12	SMP12	SMP20	SMP20	SMP28	SMP28	SMP36	SMP36
C	STD02	STD02	SMP05	SMP05	SMP13	SMP13	SMP21	SMP21	SMP29	SMP29	SMP37	SMP37
D	STD03	STD03	SMP06	SMP06	SMP14	SMP14	SMP22	SMP22	SMP30	SMP30	SMP38	SMP38
E	STD04	STD04	SMP07	SMP07	SMP15	SMP15	SMP23	SMP23	SMP31	SMP31	SMP39	SMP39
F	STD05	STD05	SMP08	SMP08	SMP16	SMP16	SMP24	SMP24	SMP32	SMP32	SMP40	SMP40
G	SMP01	SMP01	SMP09	SMP09	SMP17	SMP17	SMP25	SMP25	SMP33	SMP33	SMP41	SMP41
H	SMP02	SMP02	SMP10	SMP10	SMP18	SMP18	SMP26	SMP26	SMP34	SMP34	SMP42	SMP42

   Instructions for defining **Default Well IDs** and designing **Templates** are provided on the following pages.

Defining Default Well IDs

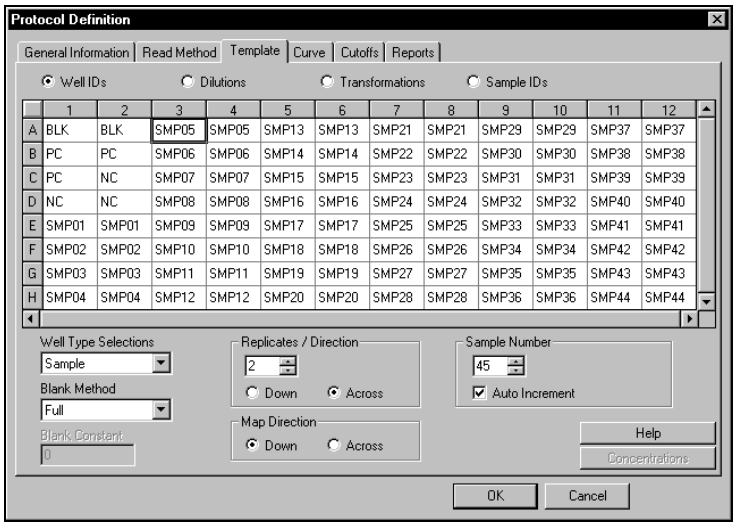
Define the default Well IDs before designing Templates.

Well IDs are used to identify the four well types supported by KCjunior – Blank, Standard, Sample, and Control. Common IDs for these well types are BLK, STD, SMP, and CTRL, respectively.

Default Well IDs are defined within the System Configuration dialog, found under **Setup|Configuration.** Define these IDs *before* creating any templates. For more information, see "Setting Up the System - System Configuration" in *Getting Started*.

Assigning Well Identifiers

Well information, such as type, location, replicates, and blanking method, is defined in the **Well ID Template.**



Note: *The parameters available within the Well ID Template for the* ***Spectral Scan reading method*** *are limited to well type selections of Blank and Sample, and Sample Numbering. One to eight wells can be scanned, and the wells do not have to be contiguous.*

Designing a New Well ID Template

Design the Well ID template before defining dilutions, transformations, or sample IDs.

To design a **Well ID Template:**

1. Ensure that the **Default Well IDs** for Blank, Standard, Sample, and Control wells are defined appropriately under **Setup|Configuration.**
2. Ensure that the correct **Plate Geometry** is selected under the Read Method tab.
3. From within the Well ID Template, select a well type from the **Well Type Selections** drop-down menu.
   - The **Well Type Selection** names are the descriptive links between the generic well type (i.e., Blank, Sample, Standard, and Control), and the 2 to 5 character **Well ID.** Well Type Selection names and Well IDs are configured under **Setup|Configuration, Well IDs.**

4. If applicable, define the **Number of Replicates, Replicate Direction,** and **Map Direction** associated with that ID:
   - Specify the **Number of Replicates** for the selected well type. The Number of Replicates value represents the number of individual replicates (1, 2, 3) within a single group (SMP, BLK, STD). KCjunior automatically increments the numeric ID suffix.
   - Specify the **Replicate Direction,** Down or Across. The Replicate Direction is the direction in which individual replicates within a single group are mapped.

     A direction of **Down** maps replicates down columns, e.g. A1-B1, C1-D1.

     A direction of **Across** maps replicates across rows, e.g. A1-A2, A3-A4.
   - Specify the **Map Direction,** Down or Across. The Map Direction is the direction in which the groups are mapped.
5. If applicable, enter the **Standard** or **Sample Number** to be associated with the starting replicate group.

   For example:
   - Setting **Standard Number** to 1 labels the starting standard group "[ID]01", such as "**STD01**".
   - Setting **Sample Number** to 6 labels the starting sample group "[ID]06", such as "**SMP06**".
6. For well types of Standard and Sample, check **Auto-Increment** to automatically increment each subsequent group number by one.
7. After selecting the Well Type and defining any additional information, **assign** the Well ID to one or more wells in the template.
   - To assign an ID to a single well, click once on the desired well.
   - To assign an ID to multiple contiguous wells, move the mouse cursor to the "starting" well, then click and hold the left mouse button. Drag the mouse cursor across and/or down to the "ending" well, then release the mouse button.
   - If the plate contains more than 8 rows and/or 12 columns, the template is viewable in **quadrants.** To select a quadrant for well ID assignment, click on the appropriate Quadrant number.

8. If the template contains **Blank** wells, select a **Blanking Method.** Available methods are Full, Half, Quarter, Row, Column, and Constant.

   If Constant is selected, set a **Blank Constant** value. The valid Blank Constant range is **-9.9999e+99** to **9.9999e+99.**

   The **Blanking Methods** are described below:

   Full — The average of the blank wells is subtracted from every well on the plate.

   Half — The average of the blank wells in the first half of the plate (column-oriented) is subtracted from each well of the first half of the plate.

   The average of the blank wells in the second half of the plate is subtracted from each well of the second half of the plate.

   Quarter — The average of the blank wells in the first quadrant of the plate (column-oriented) is subtracted from each well of the first quadrant.

   The average of the blank wells in the second quadrant is subtracted from each well of the second quadrant, etc.

   Row — The average of the blank wells in each row is subtracted from each well of that row.

   Column — The average of the blank wells in each column is subtracted from each well of that column.

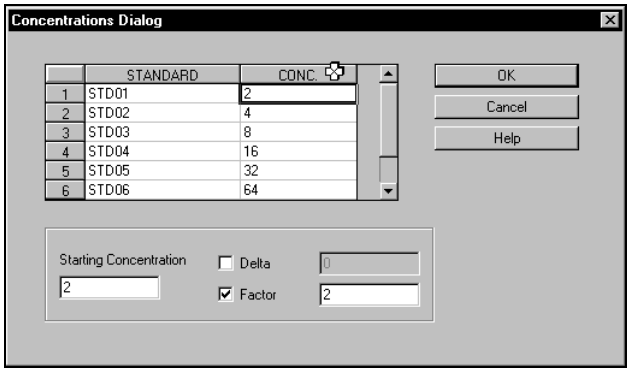
   Constant — Each well of the plate is reduced by the value of a **Blank Constant.**

9. If the template contains **Standard** wells, define the associated **Concentrations.**

   - *Before* defining concentrations, ensure that the Well ID template contains Standard well IDs as required to support the desired curve fit type. For more information, see "Defining Curve Parameters - Selecting a Curve Method" in *Defining Protocols*.

   To define **Concentrations,** click the **Concentrations** button from within the Well ID Template.

The **Concentrations** dialog appears:



- Within the **Concentrations** dialog, the Standard IDs that were defined in the template appear in sequential order in the **STANDARD** column. The concentrations associated with each Standard ID appear in the **CONC** column.
- Individual concentrations can be entered manually in the CONC column, or they can be assigned using the **Auto-Numbering** feature:

  Enter the **Starting Concentration** – the concentration value to be assigned to the first standard.

  Note: Valid concentration values include **0** and **1.0000e-99** to **9.9999e+99.**

  Choose the method by which to increase the concentration values for subsequent standards: **Delta** to increase each standard exactly by a defined value, or **Factor** to increase standards by a defined factor value.

  Enter the Delta or Factor **value,** as appropriate.

  Click the mouse button at the top of the **CONC** column to assign the **concentrations.**

  Click **OK** to save the information and return to the Well ID Template.

10. When the Well ID Template is complete, the **Dilutions, Transformations,** and **Sample ID** Templates can be designed.
    - Click the **Dilutions** radio button to assign Dilutions.
    - Click the **Transformations** radio button to define Transformation and Plate Formulas.
    - Click the **Sample IDs** radio button to assign sample identifiers.

Modifying an Existing Well ID Template

Before modifying an existing **Well ID Template,** consider the following:

- Changes made to the Well ID Template may affect the Dilution, Transformation, and Sample ID Templates as well.

  For example, if all **'PC'** wells are removed from the Well ID Template, any Transformation containing **'PC'** will become invalid, and should be modified or removed.

- Changes made to the default Well IDs may affect modifications to the Well ID Template.

  When a **Well ID Template** is created, the default Well IDs that were defined via **Setup|Configuration** are assigned to template wells, as illustrated below:

	1	2	3	4	5
A	BLK	PC	NC	SMP01	SMP02
B	BLK	PC	NC	SMP01	SMP02

  KCjunior requires that within one template, a single Well ID must be defined for wells of type Blank, Standard, and Sample. Because of this, it is useful to know if any default Well IDs have been changed before modifying an existing Well ID Template.

  In the following example, the user plans to add one more blank well to a template in a previously saved protocol. In the time since the protocol was last saved, the default well ID for wells of type Blank had been changed.

  - The original Well ID Template was created as shown above, with two wells of *type* Blank, with the *ID* **BLK.** The protocol was then saved with this template setup.
  - Via **Setup|Configuration,** a change was made to the default Well ID for wells of type Blank; they are now referred to as **BLANK** instead of **BLK.**
  - To add another Blank well, **Well Type Selection** is set to Blank, then well C1 is "clicked" to assign the ID. Now the template looks like this:

	1	2	3	4	5
A	BLK	PC	NC	SMP01	SMP02
B	BLK	PC	NC	SMP01	SMP02
C	BLANK				

  - This is currently an invalid template. To make the template valid, two options are available:

Option 1: With the Well Type Selection still set to Blank, click wells A1 and B1 to change them to **BLANK.** The template is now valid. **Note:** If any transformations in this protocol referred to "BLK", all references would have to be changed to "BLANK" to ensure validity.

Option 2: Via **Setup|Configuration,** change the default Well ID for Blank wells back to **BLK.** Return to the Well ID Template. With **Well Type Selection** set to Blank, click on well C1 to assign the ID **BLK.** The template is now valid.

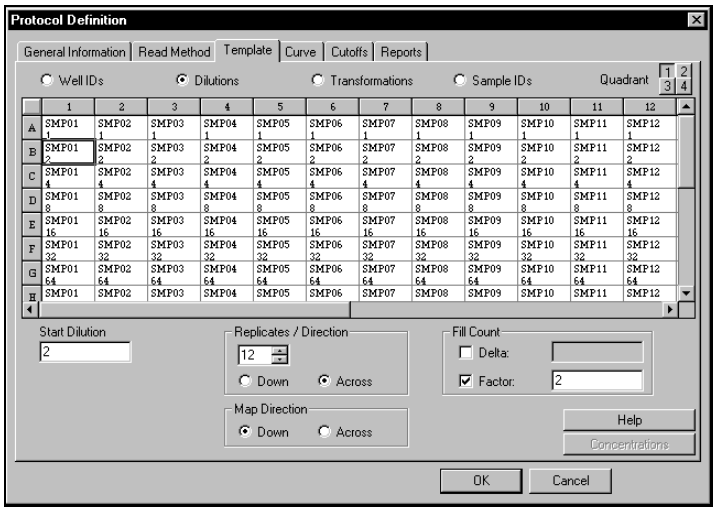
Defining Dilutions

KCjunior provides the ability to assign **dilutions** to sample and control wells.

- In **quantitative** assays, calculated concentrations are multiplied by the dilutions to produce the final concentrations.
- In **titer** assays, for each sample group containing multiple dilutions KCjunior automatically attempts to generate a curve using the dilutions as concentrations.

To define **Dilutions:**

1. Ensure that **Sample** and/or **Control** wells are defined appropriately within the **Well ID Template.**
2. Access the **Dilutions Template:**



*A dilution value of **1** indicates the sample is not diluted (1:1).*

*A dilution value of **2** indicates the sample is diluted 1:2. The resulting concentration for the sample will be multiplied by 2.0 to achieve the true concentration.*

3. To assign dilutions and **manually** increase the dilution values:
   - Enter the starting dilution value in the **Dilution** field.

     The valid dilution range is from **1.0000e-99** to **9.9999e+99.**
   - Specify the **Number of Replicates** and **Replicate Direction.**

     The **Number of Replicates** represents the number of contiguous wells to be assigned the same dilution value.

     The **Replicate Direction** is the direction in which contiguous wells should be assigned the same dilution value, **Down** or **Across.**
   - Within the template, click one or more wells to assign the current dilution value.
   - To assign the next dilution, change the value in the Dilution field, then click one or more wells.
4. To assign dilutions and let KCjunior increase the dilution values:
   - Enter the starting dilution value in the **Dilution** field.

     The valid dilution range is from **1.0000e-99** to **9.9999e+99.**
   - Specify the **Number of Replicates** and **Replicate Direction,** as explained in step 3.
   - Select a **Fill Count** method of **Delta** or **Factor.**

     Choose **Delta** to increase each dilution exactly by a defined value. Choose **Factor** to increase dilutions by a defined factor value
   - Enter a **Delta Value** or **Factor Value,** as appropriate.
   - Specify a **Map Direction** of **Down** or **Across.** This is the direction in which increasing dilutions should be mapped.
   - Highlight a block of contiguous wells to assign dilutions.
5. To **clear** any mistakes:
   - Set the **Dilution** value to 1.000.
   - Set **Replicates** to 1.
   - De-select the **Fill Count.**
   - Highlight the well(s) to be reset to 1.000.

Defining Transformations

Transformations are user-defined formulas applied to plate data for analysis or for data transformation before calculating concentrations or cutoffs.

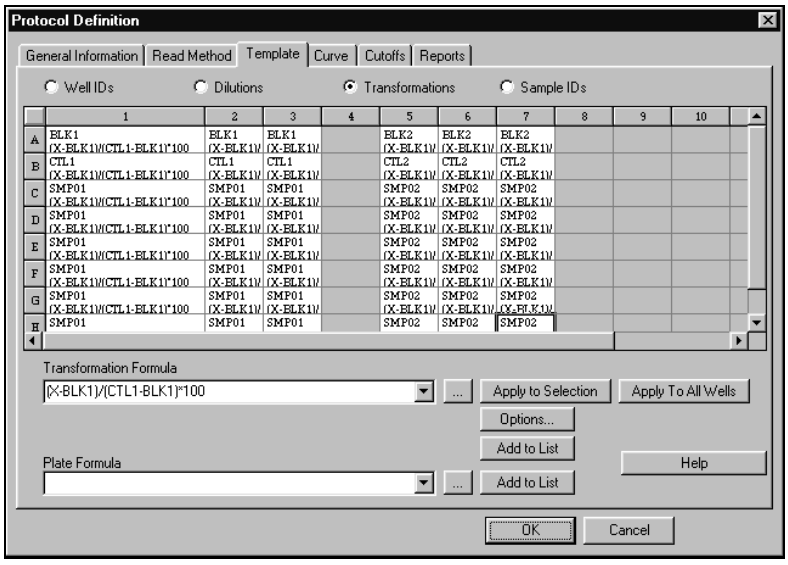
The **basis for formula calculation** may be Raw Data, Blanked Data, data normalized by Pathlength Correction, or the Maximum Slope, depending on the protocol definition. See "Essential Information" in *Getting Started* to review the results calculation structure.

Results calculation Two types of formulas can be defined within one protocol:

- **Transformation Formulas.** These formulas are manually assigned to individual wells. Results of these formulas serve as the basis for curve generation and cutoff evaluation.
- **Plate Formulas.** These formulas are automatically applied to every well on the plate that has a Well ID assigned. Results of these formulas do *not* serve as the basis for curve generation or cutoff evaluation.

To define **Transformations:**

1. Ensure that Well IDs are defined appropriately in the **Well ID Template.**
2. Access the **Transformations Template:**



Help

Click the Help button and then click the light bulb icon for tips on:

- *Making a column wider or a row higher.*
- *Removing a transformation from a well.*
- *Highlighting all wells at once.*

➢ Use this template to define, modify, and apply Transformation and Plate Formulas.

Working with Transformation Formulas

To apply an **existing** Transformation Formula to one or more wells:

1. To the right of the **Transformation Formula** field, click the down arrow to see a list of available formulas.
2. Click on the desired formula to select it.
3. To apply the formula to all wells, click the **Apply To All Wells** button.

4. To apply the formula to specific wells, use the mouse to highlight one well or a block of wells, then click the **Apply to Selection** button.

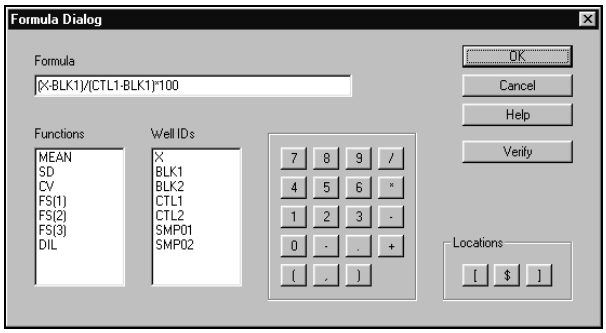
5. To access **Copy, Paste,** and **Undo** features, click either the right mouse button or the **Options** button.

To define and apply a **new** Transformation Formula:

1. Type the formula in the **Transformation Formula** field. See "Formula Syntax" at the end of this section for more information.

   Alternatively, let KCjunior assist with defining a new Transformation Formula:

   ➢ Click the button to the right of the Transformation Formula field to access the **Formula** dialog:



See "Formula Syntax" for information on mathematical values and symbols, and location indicators.

➢ Assemble the formula using a combination of Functions, Well IDs, Mathematical values and symbols, and Location indicators.

A **Function** performs a specific operation on the contents inside the parenthesis, such as the calculation of a mean value or the Standard Deviation.

The **Well IDs** list contains those Well IDs that are valid for this protocol. Each Well ID represents the mean value of its group (CTL1 represents the mean of all wells labeled as "CTL1"). The identifier **X** represents the current value of the well.

- **Functions** and **Well IDs** can be entered manually, or they can be selected from the list boxes.

  To *add* an item from a list box to the Formula field, highlight the desired item then double-click.

  To *insert* an item between two existing items in the Formula field, begin by clicking the mouse once in the Formula field where the new item should appear. Highlight the desired item in the list box, then double-click to insert.

- **Mathematical values** and **symbols** can be entered manually, or selected by clicking the appropriate buttons.

- **Location indicators** can be entered manually, or selected by clicking the appropriate buttons.

- KCjunior provides the option to perform a verification of the formula and check for syntax errors. When the formula is fully assembled, click the **Verify** button.

  If the test is *successful*, the message "Formula is valid" appears.

  If the test is *unsuccessful*, a message will appear describing the error(s). Edit the formula accordingly, then click **Verify** again.

- Click **OK** to close the Formula dialog and return to the Transformations Template. The newly created formula will appear in the **Transformation Formula** field.

2. (Optional) Click the **Add to List** button to save the new Transformation Formula..

3. To apply the formula to *all* wells, click the **Apply to All Wells** button.

To apply the formula to *specific* wells, use the mouse to highlight one well or a block of wells to which the formula should be applied, then click the **Apply to Selection** button.

KCjunior performs basic formula validation at this time. For example, if a formula references a well that is invalid for the current plate geometry, a formula validation error message appears.

To perform a more thorough validation, highlight the well containing the formula in question, open the **Formula** dialog, then click the **Verify** button.

4. To access **Copy, Paste,** and **Undo** features, click either the right mouse button or the **Options** button.

Working With Plate Formulas

To apply an **existing** Plate Formula:

1. To the right of the **Plate Formula** field, click the down arrow to see a list of available formulas.
2. Click on the desired formula to select it.
   - During data reduction, this formula is automatically applied to every well that has been assigned a Well ID.

To define and apply a **new** Plate Formula:

1. Type the formula in the **Plate Formula** field.

   See "Formula Syntax", "Order of Precedence", and "Examples" later in this section for more information.

   Alternatively, let KCjunior assist with defining a new **Plate Formula.**

   - Click the button next to the Plate Formula field to access the **Formula** dialog.

     See "To define and apply a new Transformation Formula" under *Working with Transformation Formulas* for instructions.

2. (Optional) Click the **Add to List** button to save the new Plate Formula.

Formula Syntax

In KCjunior, a formula contains a combination of Functions, Well IDs, Mathematical values and symbols, and Location indicators. Formula elements are listed and described below:

+

The **+** operator represents addition.

- **PC + NC** calculates the sum of the PC mean and the NC mean.
- **X + 0.005** calculates the sum of the current well and 0.005.

-

The **-** operator can represent either subtraction or negation.

- **PC - BLK** subtracts the BLK mean from the PC mean.
- **X-BLK** subtracts the BLK mean from the current well.

*****

The * operator represents multiplication.

- **SMP01*10** multiplies the mean of the sample replicate group SMP01 by 10.
- **X*100** multiplies the current well by 100.

/

The **/** operator represents division.

- **PC/NC** divides the PC mean by the NC mean.
- **X/[A2]** divides the current well by the value in well A2.

( )

Parentheses **( )** represent an inclusive unit for calculation.

- **(PC-NC)/100** divides the difference between the PC mean and the NC mean by 100.
- **([B4]/STD1)*100** divides well B4 by the STD1 mean, then multiplies the result by 100.

CV( )

The **CV** function calculates the coefficient of variation of the contents inside the parenthesis. The CV represents the standard deviation as a percentage of the mean: (standard deviation/mean)*100.

The CV function supports up to five parameters, each of which must equate to a single value.

- **CV(3.124, 3.094, 2.997)** returns 2.161.
- **CV(CTRL1, CTRL2)** returns the coefficient of variation for all replicates of both control groups.

If there is a single parameter inside the parenthesis then it is assumed to be a Well ID.

- **CV(PC)** returns the coefficient of variation for the group PC.

DIL

The **DIL** function brings forth the currently assigned dilution value in the well. This value can then be included in a formula.

The **DIL** function has no parameters associated with it. It returns a single value, the dilution defined for the well in the Dilutions template.

- **DIL** returns the currently defined dilution value for the well, such as 10.000.
- **DIL * X** multiplies the defined dilution value by the current value in the well. For example, with a quantitative assay, the X value may represent the calculated concentration for the well.
- **DIL** is not valid within a **Cutoff** formula.

FS( )

The **FS** function is available for fluorescence and luminescence protocols, and is used to specify a particular **filter set** for use in the formula.

- **FS(4) * 100** multiplies the result in each well of Filter Set #4 by 100.
- **FS(1) / FS(2)** divides the result in each well of Filter Set #1 by the result in the corresponding well of Filter Set #2.
- To control the basis for standard curve and concentrations calculation in a **multiple filter set** read, apply **FS(<filter set number>)** to every well in the Transformation Template.

  KCjunior automatically uses the results calculated for that filter set when generating the curve and calculating concentrations.
- **FS** is not valid within a **Cutoff** formula.

MEAN( )

The **MEAN** function calculates the average of the contents inside the parenthesis. It supports up to five parameters, each of which must equate to a single value.

- **MEAN(7, 16, 4)** returns 9.
- **MEAN(PC, NC)** returns the mean of the PC and the NC replicate groups.

If there is a single parameter inside the parenthesis then it is assumed to be a Well ID.

- **MEAN(BLK)** returns the mean of the BLK wells.

SD( )

The **SD** function calculates the standard deviation of the contents inside the parenthesis. The Standard Deviation is a measure of the distribution of individual values relative to the mean.

The SD function supports up to five parameters, each of which must equate to a single value.

- **SD(3.124, 3.094, 2.997)** returns 0.06638.
- **SD(CTRL1, CTRL2)** returns the standard deviation for all replicates of both control groups.

If there is a single parameter inside the parenthesis then it is assumed to be a Well ID.

- **SD(NC)** returns the standard deviation of the group NC.

WV( )

The **WV** function references the set of data at the wavelength specified inside the parenthesis.

- **WV(450)** references the set of data read at or calculated for 450 nm. (In a MultiWavelength read, if an empty plate is read at 450 nm, WV(450) represents the **Result,** or the difference in readings between the actual microplate and the empty plate).
- **WV(405)/WV(630)** in a well divides its 405 nm result by its 630 nm result.
- To control the basis for standard curve and concentrations calculation in a **MultiWavelength** read, apply **WV(<wavelength>)** to every well in the Transformation Template.

  KCjunior automatically uses the measurements at that wavelength when generating the curve and calculating concentrations.
- **WV** is not valid within a **Cutoff** formula.

[ ]

Use brackets **[ ]** to reference a well by its location on the plate. For example:

- (([A2]-[A12])/2)+[A12]
- X-([C2]-[C3])

$

Use a dollar (**$**) sign to retain the row and/or column reference when **copying** formulas from cell to cell in the template.

- [A1], [$A1], [A$1], and [A1] all refer to well A1.
- [A1] copied from A1 to B1 becomes [B1].
- [$A1] copied from A1 to B2 becomes [$A2].
- [A$1] copied from A1 to B2 becomes [B$1].

X

The letter **X** represents the value of the current well.

- **X-BLK** in well G6 subtracts the BLK mean from the value in well G6.
- **X-[A2]** in well A1 subtracts the value in well A2 from the value in well A1.
- **X** is not valid within a **Cutoff** formula

<well id>

Any **Well ID**entifier used in a formula represents the group mean or average.

- **PC** represents the mean of all PC wells on the plate.
- **SMP01** represents the mean of all SMP01 wells on the plate.
- **SD(BLK)** calculates the standard deviation of the BLK wells.

Order of Precedence

KCjunior processes formulas according to a certain **order of precedence:**

1st: ( ) parenthesis

2nd: - unary minus

3rd: * and /

4th: + and -

- Operators * and / are of equal precedence
- Operators + and - are of equal precedence
- Within an expression, operators of equal precedence are processed from left to right

Examples:

Values in three PC wells: 3.002, 3.186, 2.998

Values in three NC wells: 0.868, 0.798, 0.824

(PC-NC)/100 = 0.02232

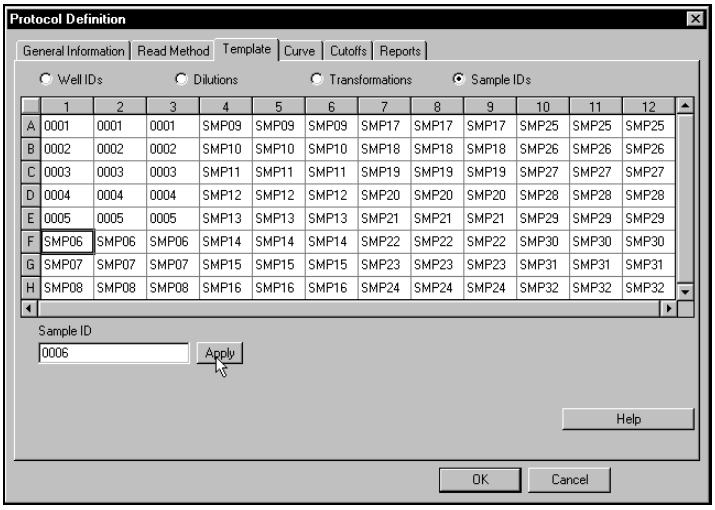
(MEAN(PC,NC))/100 = 0.01946

Identifying Samples

KCjunior provides the ability to assign custom identifiers, such as "Jones" or "003672865A", to sample groups. These identifiers are referred to as **Sample IDs,** and they are defined within the **Sample ID Template.**

To define **Sample IDs:**

1. Ensure that **Sample** wells are defined appropriately within the **Well ID Template.**
2. Access the **Sample ID Template:**



3. In this template, highlight a sample well to prepare it for identification. The well ID will appear in the Sample ID field.

   Only one replicate within a sample group needs to be highlighted.

4. Replace the contents of the **Sample ID** field with the new sample group identifier, using up to 16 alphanumeric characters.

5. Click the **Apply** button to assign the ID to the highlighted sample group.
6. Repeat for the remaining sample wells.

Curves

KCjunior provides three methods for generating curves:

- A **standard curve** can be generated based on the standard wells defined within the protocol.
- A previously generated and **stored curve** can be referenced from within the protocol.
- A **titer curve** can be generated using multiple dilutions of sample groups as concentrations.

The **basis for curve calculation** may be Raw Data, Blanked Data, data normalized by Pathlength Correction, or Maximum Slope, depending on the protocol definition. See "Essential Information" in *Getting Started* to review the results calculation structure.

To define **Curve** parameters:

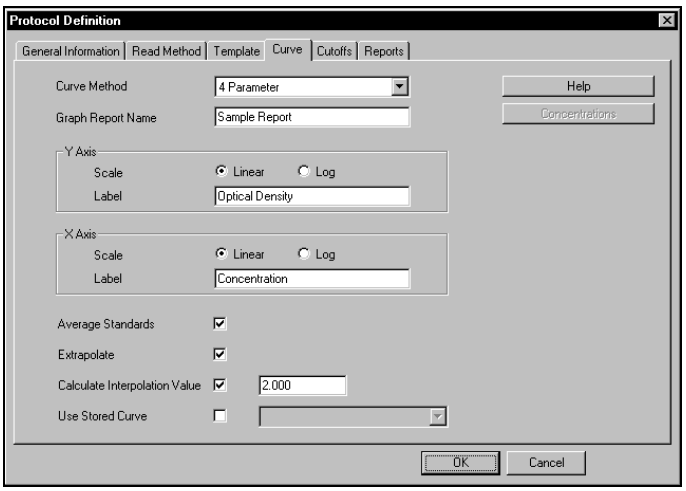
1. If this is a **titer** protocol, ensure that samples and dilutions have been defined.

   If the curve will be based on standards, ensure that the Standard well IDs are assigned within the **Well ID Template.**

   Note: Each curve-fitting method requires a **minimum** number of standards:

Linear	2	2 Parameter	4
Quadratic	3	4 Parameter	4
Cubic	4	Point-to-Point	2
		Cubic-Spline	3

2. Access the **Curve** dialog:



3. If the standard curve will be based on a stored curve:
   - Click **Use Stored Curve** at the bottom of the Curve dialog.
   - Select a stored curve from the drop-down list.
   - No other Curve parameters need to be defined.
4. Select a **Curve Method** from the drop-down list.

   Available options are Linear, Quadratic, Cubic, 2 Parameter, 4 Parameter, Point-to-Point, and Cubic Spline:

   Linear: A simple best-fit, straight line is plotted using the values of the standards.

   Quadratic: Uses the quadratic equation $ax^2+bx+c=y$ to plot the standard values. Using this curve, any particular data point for a standard which deviates from the ideal value does not affect the entire curve.

   Cubic: Uses the equation $ax^3+bx^2+cx+d=y$ to plot the standard values. The cubic curve is affected even less than the quadratic fit when any particular standard has a poor value.

   2 Parameter: Also known as **Logit-Log.** Characterized by a skewed sigmoidal plot that eventually becomes asymptotic to the upper and lower standard values. The logistic equation is algebraically transformed to a simpler form, where experimentally determined values are used for the responses at concentrations of zero and infinity.

   4 Parameter: Characterized by a skewed sigmoidal plot that eventually becomes asymptotic to the upper and lower standard values. The four parameters are left asymptote, right asymptote, slope, and slope at the inflection point.

   Point-to-Point: Follows each standard point with no averaging of the values to "smooth" the curve.

   Cubic Spline: A piecewise polynomial approximation consisting of joining a set of data points by a series of straight lines, which is then "smoothed" by using a cubic fit.
5. Enter a name for the graph in the **Graph Report Name** field, using up to 16 alphanumeric characters.
   - This name appears on Standard Curve reports.
6. Select a **Y Axis Scale** of **Linear** or **Log.**
   - For most curve methods, if a **logarithmic** scale is selected, then logarithmic numbers are used for both the calculation of the curve and the plotting of the curve.

     For the **2 Parameter** and **4 Parameter** curve methods, however, the logarithmic scale is applied only to the plotting of the curve. The curve is calculated using linear numbers.

7. Specify a **Y Axis Label,** using up to 32 alphanumeric characters. This label appears on displayed and printed reports.
   - Common labels are “Optical Density”, “RFU”, and “RLU”.
8. Select an **X Axis Scale** of **Linear** or **Log.**
   - For most curve methods, if a **logarithmic** scale is selected, then logarithmic numbers are used for both the calculation of the curve and the plotting of the curve.

     For the **2 Parameter** and **4 Parameter** curve methods, however, the logarithmic scale is applied only to the plotting of the curve. The curve is calculated using linear numbers.
9. Specify an **X Axis Label,** using up to 32 alphanumeric characters. This label appears on displayed and printed reports.
   - A common label is “Concentration”.
10. If the Standards are defined with *multiple* replicates, enable standard averaging by clicking **Average Standards.**
    - If **Average Standards** is *enabled*, KCjunior uses the average of the standard replicates when generating the curve.
    - If **Average Standards** is *disabled*, KCjunior uses the individual standard replicates when generating the curve.
    - **Note:** The Point-to-Point and Cubic Spline curve methods *automatically* use the average of the standard replicates.
11. If required, click **Extrapolate** to display extrapolated concentrations (unknown values on the Y axis which are outside the range of the standards).
    - On data displays and reports, extrapolated concentrations are enclosed by parenthesis, for example **(27.345).**
    - **Note:** The Cubic Spline curve method does not permit the display of extrapolated concentrations.
12. (Optional) To calculate the concentration for a specific value, enable **Calculate Interpolation Value,** then enter the desired value.

    Valid Interpolation Values include **0** and **1.0000e-99** to **9.9999e+99.**
    - If a curve generates successfully, KCjunior calculates a concentration for the entered Interpolation Value. The Interpolation Value is displayed on the Standard Curve dialog and on the Standard Curve report.

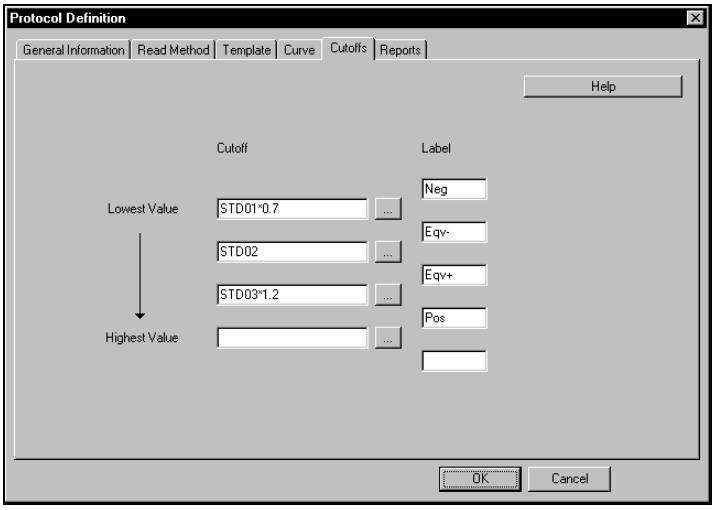
Cutoffs

Cutoffs are used to classify results. During data reduction, KCjunior evaluates results against user-defined cutoff criteria and assigns each well a user-defined **Label.**

The **basis for cutoff evaluation** may be Raw Data, Blanked Data, data normalized by Pathlength Correction, or Maximum Slope, depending on the protocol definition. See "Essential Information" in *Getting Started* to review the results calculation structure.

To define **Cutoffs** and **Labels:**

1. If a cutoff value or formula contains references to specific Well IDs, ensure that the IDs are defined in the Well ID Template.
2. Access the **Cutoffs** dialog:



3. Enter the cutoff values or formulas in the **Cutoff** fields. These are the criteria against which the plate data will be evaluated.

*See "Data Reduction and Analysis" under **Essential Information** to review the calculation structure which determines the basis for cutoff evaluation.*

- Up to **four** cutoffs can be specified. Each cutoff can be any one of the following:

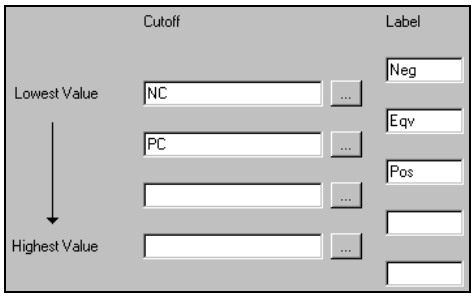
  A simple **numeric value,** such as 1.500 or 50000.

  A **well ID,** such as NC or LPC.

  A **formula** combining numeric values and well IDs, such as STD01*0.800.

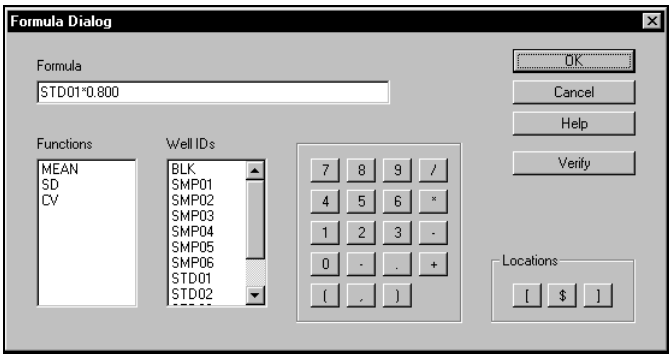
- Multiple cutoffs must be entered in *ascending* order, *down* the screen.

  In the following example, the NC mean must equate to a value that is less than that of the PC mean for the cutoff evaluation to be valid.



4. Click the ... button to the right of a Cutoff field to let KCjunior assist with formula definition and verification.

   The **Formula** dialog appears:



   - Assemble the formula using a combination of Functions, Well IDs, Mathematical values and symbols, and Location indicators.
   - **Functions** and **Well IDs** can be entered manually, or they can be selected from the list boxes.

     A **Function** performs a specific operation on the contents inside the parenthesis, such as the calculation of a mean value or the Standard Deviation.

     The **Well IDs** list contains those Well IDs that are valid for this protocol. Each Well ID represents the mean of its replicate group.

     To *add* an item from a list box to the Formula field, highlight the desired item then double-click.

     To *insert* an item between two existing items in the Formula field, begin by clicking the mouse once in the Formula field where the new item should appear. Highlight the desired item in the list box, then double-click to insert.

- **Mathematical values** and **symbols** can be entered manually, or selected by clicking the appropriate buttons.
- **Location indicators** can be entered manually, or selected by clicking the appropriate buttons.
- When the formula is fully assembled, click the **Verify** button to check for syntax errors.

  If the test is *successful*, the message "Formula is valid" appears.

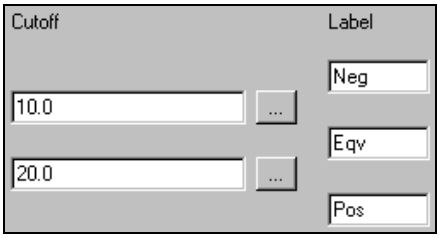
  If the test is *unsuccessful*, a descriptive message appears. Edit the formula accordingly and click **Verify** again.
- Click **OK** to close the Formula dialog and return to the Cutoff dialog. The newly created formula will appear in the appropriate **Cutoff** field.

5. Specify the text to be associated with each cutoff zone in the **Label** fields. These labels will be assigned to wells after evaluation.
   - Each label can contain up to **five** alphanumeric characters.
   - The number of labels must **exceed** the number of cutoff values or formulas by one.
   - In the following example, labels will be **applied** to wells as follows:

     Neg if the value in the well is less than 10.0.

     Eqv if the value in the well is greater than or equal to 10.0 and less than 20.0.

     Pos if the value in the well is greater than or equal to 20.0.



Reports

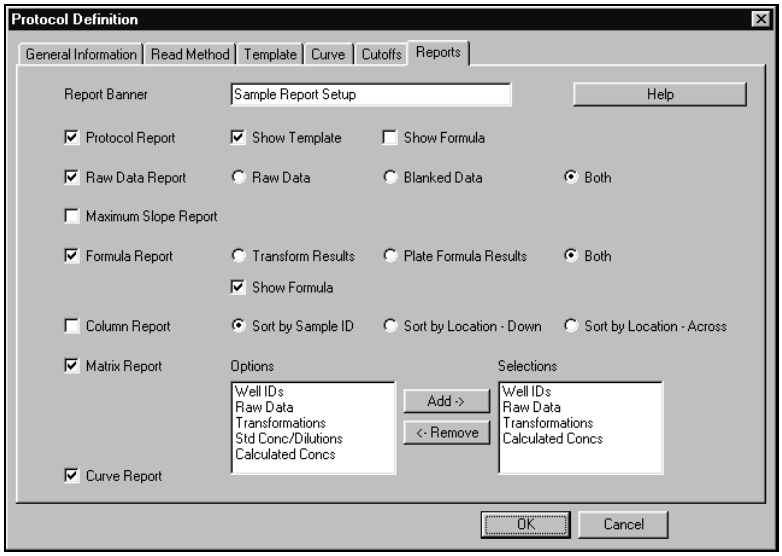
KCjunior provides up to seven different reports, depending on the current protocol settings. The contents of some of these reports can be further customized for greater flexibility with data presentation.

To select the reports for use with a particular protocol:

1. Choose to modify an existing protocol or create a new one.
2. Select the **Reports** tab. The Reports dialog appears.

*Appendix A at the end of the User's Guide contains **sample** reports.*

*Most reports require that well identifiers be assigned in the template. The **Raw Data Report,** however, can be printed even if no well ID's are assigned.*



3. Define a **Report Banner,** using up to 32 alphanumeric characters. This banner appears at the top of each printed report.
4. Check the box or radio button next to every report and corresponding option(s) to be printed.
   - The **Protocol Report** lists many of the parameters defined in this protocol.

     Click **Show Template** to include a row x column matrix containing all defined Well IDs.

     Click **Show Formula** to list the Transformation and/or Plate Formulas.
   - The **Raw Data Report** contains the raw and/or blanked optical density data or relative fluorescence/luminescence data in one or more matrices corresponding with the plate geometry.

     Click **Raw Data, Blanked Data,** or **Both,** as appropriate.

For **MultiWavelength** reads, one matrix prints for each wavelength.

For **Fluorescence** and **Luminescence** reads, one matrix prints for each filter set.

- The **Maximum Slope Report** is available with kinetic protocols. It reports the maximum slope calculated for every well, in a matrix corresponding with the plate geometry.

  For **Fluorescence** and **Luminescence** kinetic protocols with two filter sets defined, two maximum slope values will print for each well (one for each filter set).

- The **Formula Report** contains the results of the Transformation Formula(s) and/or Plate Formula in a matrix corresponding with the plate geometry.

  Click **Transformation Formula, Plate Formula,** or **Both,** as appropriate.

  Click **Show Formula** to list the Transformation and/or Plate Formulas.

- The **Column Report** lists Sample IDs/Well IDs, well location, up to three categories of measurement values, and assigned cutoff labels, if appropriate.

  Information can be sorted by Sample ID or by location in the plate (down or across).

  If sorting is by Sample ID, the report also contains the mean, standard deviation, and coefficient of variation for each Sample ID / Well ID.

- The **Matrix Report** contains well-specific information in a matrix corresponding with the plate geometry.

  The contents of this report can be customized:

  In the **Options** box, highlight up to 8 items to appear on the report, then click the Add button. The selected item(s) will appear in the Selections box.

  Available options depend on the current protocol settings. See "Selecting Options for the Matrix Report" on the next page for more information.

  To *remove* an item from the Selections box, highlight the item and click the **Remove** button.

  To *insert* a reporting option between items in the Selection box, highlight the item in the Selection box above which the new option will be inserted, highlight the data set in the Options box, then click **Add.**

- The **Curve Report** contains the graphical representation of the standard, titer, or spectral scan curve, as appropriate for the protocol.

5. Select **OK** to close the protocol and retain the new settings, or click another tab to define additional information.

- The contents of some of these reports can be further customized for greater flexibility with data presentation.
- Appendix A of the "User's Guide to KCjunior" contains **samples** of each report type.
- Most reports require that well identifiers be assigned in the template. The Raw Data Report, however, can be printed even if no well ID's are assigned.

Selecting Options for the Matrix Report

The options available for printing depend on the current protocol settings, and can include some or all of the following:

- **Well ID**
- **Raw, Blanked, and/or Corrected Data**
  - Endpoint and Kinetic absorbance reads: The options to include raw or blanked data in the Matrix Report are presented as "Raw Data" or "Blanked Data".
  - Endpoint and Kinetic fluorescence/luminescence reads: There are multiple "Raw" and "Blank" options available for the Matrix Report, one for each filter set defined.

    For example, if the Read Method options specify 3 filter sets, and there are blank wells defined in the template, the selectable Matrix Report options include the following:

    Raw(1)
    Raw(2)
    Raw(3)
    Blank(1)
    Blank(2)
    Blank(3)

    Raw(1) represents the raw data values obtained with filter set 1, Blank(1) represents the values obtained with filter set 1 after blank subtraction, and so on.

- MultiWavelength absorbance reads: An option is presented for each individual wavelength. For example, if the protocol specifies reading at 405, 450, and 490 nm, with blanking, the options include the following:

  Raw(405)
  Raw(450)
  Raw(490)
  Blank(405)
  Blank(450)
  Blank(490)

  If *Read Empty Plate* is enabled for a wavelength, the Raw and Blanked data sets for that wavelength represent the results after empty plate subtraction.

  If *Pathlength Correction* is enabled, the "Corr" option is presented for each wavelength. This data set represents results after blank subtraction and pathlength correction.

- **Pathlengths**
  - The Pathlengths option is available for MultiWavelength protocols with Pathlength Correction enabled.
- **Max Slope**
  - The Max Slope option is available for Kinetic protocols, to report the maximum slope calculated for every well.
  - For fluorescence/luminescence Kinetic protocols with two filter sets defined, Max Slope (1) and Max Slope (2) represent the maximum slopes calculated for every well, for filter sets #'s 1 and 2, respectively.
- **Transformations**
- **Plate Transformations**
- **Standard Conc/Dilutions**
  - Standard concentrations or dilutions are included as they are defined in the protocol, under the Curve tab.
- **Calculated Concentrations**
- **Cutoff Labels**

Reading Plates

Overview

The terms “plate data” and “results” refer to the set of measurements either retrieved from a microplate reader, entered manually by the operator, or read from an existing data file, and the results of any calculations performed on those measurements.

Reading Plates describes how to use KCjunior to:

- Read a microplate with, and retrieve plate data from, the attached reader.
- Simulate a plate read.
- Copy data from a spreadsheet and paste it into a KCjunior plate.
- Import plate data from an archive results database into the current results database.
- Share plate data and results databases among multiple KCjunior users.
- Read data from a KCJr for DOS plate file or from an Omni-System absorbance file.
- Export plate data to a text file or directly into a Microsoft Excel spreadsheet.
- View, store, and access results.

Working With Plates and Results

This section describes accessing, generating, modifying, storing, and sharing plate data.

Where is Plate Data Stored?

Data for each plate is stored in a **Results Record** in the **Results Database.** Each record is identified by a user-assigned **Results ID.**

One results record stores all of the raw optical density, fluorescence, or luminescence values for a plate that was read once (as with a single-plate endpoint protocol), or for a plate that was read multiple times (as with a kinetic, multi-wavelength, spectral scan, or multi-plate endpoint protocol).

Results Databases are typically stored on a computer's hard drive, or on a network to which the computer is attached. When opening or saving results records, KCjunior automatically refers to the **Results Database Pathname** for retrieval and storage.

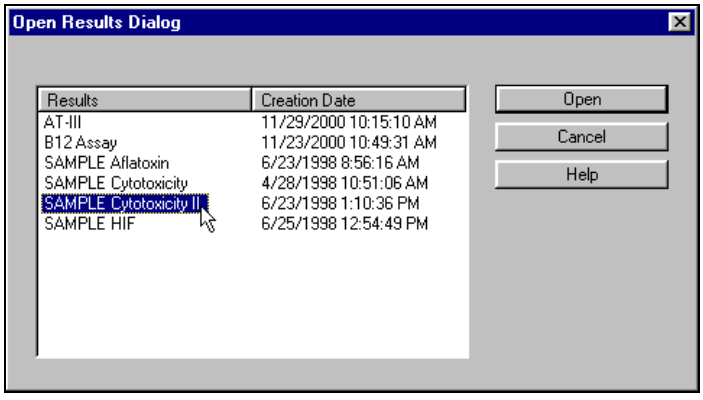
- The Results Database Pathname represents the **location** and **name** of the "current" results database.
  - Example pathname: C:\KCjunior\Database\Results.mdb.
- The results database pathname can be viewed or changed under **Setup|Configuration, Pathnames.**

Opening an Existing Results Record

To open an existing results record, begin by clicking the **Open Results** button in the KCjunior main screen, selecting **Results|Open Results,** or by selecting a name from the Results drop-down menu in the toolbar.

The **Open Results** dialog appears, as shown below:

*Click on the **Results** column header to sort the list by name, in ascending or descending order. Or click on the **Creation Date** column header to sort by creation date and time.*



- To **open** an existing results record, highlight the desired **Results** record name in the list, then double-click the mouse button or click the **Open** button.
- To change the **sort** order, click on the Results column header and/or the Creation Date column header.
- To **close** this dialog without opening a record, click **Cancel** or press the **Esc** key.

Generating a New Set of Plate Data

Plate data can be retrieved from a microplate reader, generated manually (referred to as *simulation data*), or read from a file.

To generate any new set of plate data, begin by clicking on the **Read Plate** button in the KCjunior main screen, selecting **Results|Read Plate,** or by clicking the **toolbar icon.**

The **Read Plate** dialog appears, providing three options for generating the plate. For more information see:

- "Reading a Plate" on page 6-7
- "Simulating a Plate Read" on page 6-12
- "Reading Plate Data from a File" on page 6-13

Before Reading a New Plate

A microplate can be read with or without a **Protocol.** A protocol contains information for the operation of the microplate reader, as well as instructions for analyzing data retrieved from the reader.

To read a plate without a protocol (to perform a **fast read**), simply click the **Read Plate** button from within the Read Plate dialog. KCjunior requests minimal read method information then initiates the plate read. If desired, a protocol can be opened or defined after the data is retrieved from the reader.

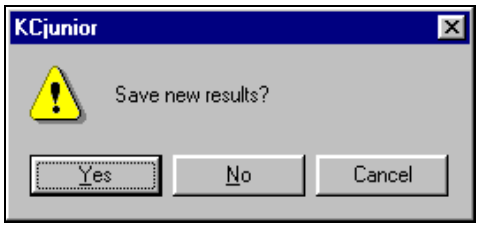
To define or open a protocol before reading a plate, refer to the *Defining Protocols* section of this User's Guide.

Saving and Closing Results Records

After generating or modifying a plate, save the information by selecting **Results|Save Results** or by clicking the toolbar icon. Results records are stored in the current **Results Database.**

KCjunior either automatically saves the record under the **Results ID** assigned prior to microplate reading, or requests the entry of a Results ID if not previously assigned.

To *close* an open record, select **Results|Close Results** or click the toolbar icon. If the record is new, or if modifications were made to an existing record, KCjunior provides an option to save:

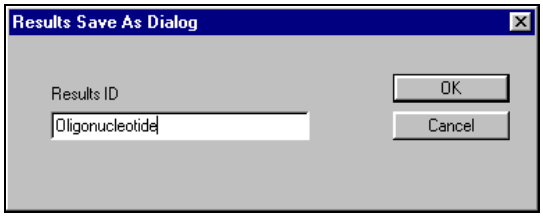


- Click **Yes** to save the new or modified information and close the record.
- Click **No** to close the record without saving changes.
- Click **Cancel** to close this dialog and return to the main view. The new or modified information is retained but not yet saved.

Copying an Existing Results Record

KCjunior provides an option to copy all of the contents of an existing results record to a new record with a different name.

To copy an existing record, begin by opening it. Once it is open, immediately select **Save As** from the Results Menu. KCjunior asks for a new name:



- Enter a new name, then click **OK** to create the new results record, save it in the current Results Database, and keep it open for modification.
- Click **Cancel** to cancel this operation and return to the original results record.

Sharing Results Records and Databases

Since KCjunior stores results records in a database, there are several options for **sharing** records and databases among users:

- A results database can be stored on a **network** for multi-user availability.
  - Each user must configure the **Results Database Pathname** within his or her copy of KCjunior to point to the network database.
- A results database can be copied to a diskette or CD and exchanged between users.

  If the database has grown too large to fit on a diskette, create an empty results database, then copy only the desired results records into the new database:

  1. Select Utilities|Database Maintenance|**Create Archive Results Database.** The Create Empty Results Database dialog appears.
  2. In the **Save in** field select a location for the new database, then enter an identifying **File** name.
  3. Click **Save** to create the database and return to the main screen.
  4. Select Utilities|Database Maintenance|**Archive Results.** The **Archive Results Database Pathname** dialog appears.
  5. Enter the drive, directory, and file name for the newly created empty database.
  6. Click **OK.** The **Archive Results** dialog appears, listing the contents of the current Results Database.
  7. Select the record(s) to be copied. Do NOT check **Delete after archive.**
  8. Click the **Archive Results** button. The selected records are copied to the new database.
- Results records can be **imported** from one database into another. See **Importing Results** below for more information.

  Suggestions:
  - Results records can be imported from a "master" results database stored on a network.
  - Results records can be imported from a results database stored on a diskette.

Importing Results

Results records from one results database can be **imported** into another results database. The Results Database that will *receive* the imported records is the one defined under **Setup|Configuration|Pathnames.**

To import results records from a stored database:

1. If a results record is currently open, close it.
2. Select **Results|Import Results.** The **Import Results Database Pathname** dialog appears.
3. In the **Results Database Pathname** field, enter the pathname (drive, directory, and file name), of the database containing the records to be imported.

   Alternatively, use the **Browse** feature to locate and select the database.
4. Click **OK.** The **Import Results** dialog appears, listing all records in the database.
   - Click the **Results** and **Creation Date** column headers to sort the records alphabetically or by date.
5. Use the mouse to select the record(s) to import.
   - Press the **Shift** key while using the mouse to select contiguous records.
   - Press the **Ctrl** key while using the mouse to select non-contiguous records.
6. Click the **Import Results** button. KCjunior imports the selected records.
   - If a duplicate record name is detected, KCjunior does **not** overwrite the record.

Reading a Plate

A microplate can be read with or without pre-defining or opening a **Protocol.** A protocol contains information for the operation of the microplate reader, as well as instructions for analyzing data retrieved from the reader.

Reading a Plate Without a Protocol

If a "fast read"is required (that is, with no protocol open), KCjunior requests minimal information then initiates the plate read. Following the read, a protocol can be created or opened to analyze the data.

Note: The **spectral scan** read method requires a protocol to define the wells to be scanned.

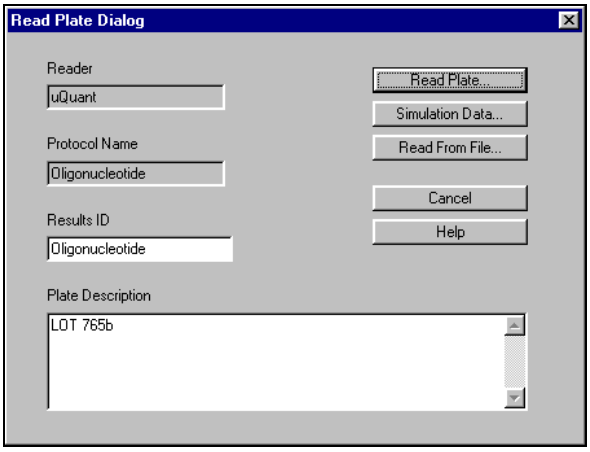
To perform a **Fast Read:**

1. Ensure that the reader is properly attached to the computer, and that the correct reader type and communication parameters are defined under **Setup|Reader.**

   See "Setting up the System" in *Getting Started* for more information.

2. Initiate the read by clicking the **Read Plate** button in the KCjunior main screen, selecting **Results|Read Plate,** or by clicking the **toolbar icon.**

   The **Read Plate** dialog appears:



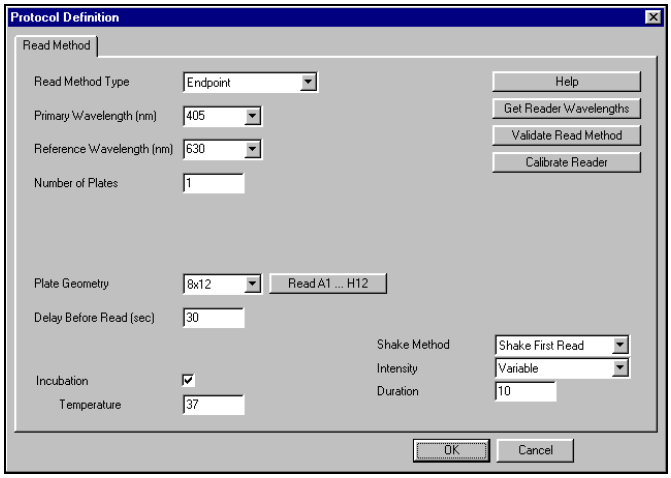
3. Enter a **Results ID,** using up to 32 alphanumeric characters. The ID is optional in this dialog, but it will be required later if the results are to be stored in the Results Database.

   - If performing a **multiple-plate endpoint read,** all of the plates in the read will be stored in the Results Database (in one record) under this Result ID.

4. (Optional) Enter a **Plate Description,** using up to 1024 alphanumeric characters.

5. Click the **Read Plate** button.

   The **Read Method** dialog appears:

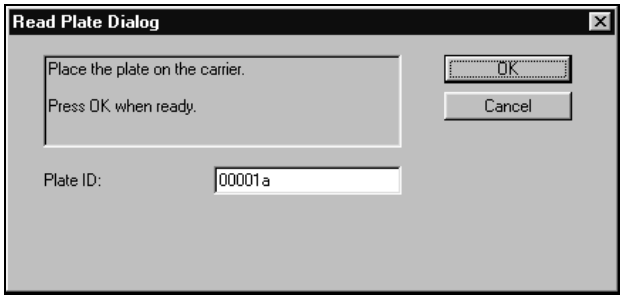


6. Define the basic **Read Method** parameters. See "Defining Read Method Parameters" in *Defining Protocols* for more information.

7. (Optional) If the attached reader supports calibration, click the **Calibrate Reader** button to calibrate the reader at the defined wavelength(s).

8. Click **OK** to continue with the read.

   A second **Read Plate** dialog appears.



9. (Optional) Manually assign a **Plate ID** to this plate.
   - For **multiple plate endpoint absorbance protocols,** each individual plate can have its own Plate ID assigned. This prompt appears before each plate is read.
10. Place the plate on the carrier, then click **OK** to initiate the plate read.
    - If the attached reader is equipped with a barcode scanner *and* **Use Reader's Barcode Scanner for Plate ID** is enabled in KCjunior under Setup|Configuration|General, KCjunior attempts to scan the plate. If no barcode is detected, or if the attached reader is not equipped with a barcode scanner, the Plate ID can be entered manually.
    - Click the **Abort Read** button to halt the read in progress. Data reduction is based on any data retrieved up to the time the button was clicked.
    - When the plate read is complete, KCjunior automatically retrieves the measurements from the reader, performs data reduction, and displays the results. See *Viewing Results* or *Interpreting Results* for more information.
11. To **save** the results:
    - Select **Results|Save Results,** or press **CTRL+S.**

      KCjunior requests a **Results ID** if one was not defined before the plate read. Enter the ID using up to 32 alphanumeric characters. This is the name under which the record will be stored in the Results Database.

      Click **OK** to save the results.
12. To apply a **protocol** to the results, leave the results record open and do one of the following:
    - **Open an existing protocol.** If the read method parameters are compatible, KCjunior automatically recalculates the results. If the read method parameters are not compatible, KCjunior provides further instructions.
    - **Create a new protocol.** KCjunior automatically transfers the read method parameters defined for the fast read into the new protocol.

Reading a Plate With a Protocol

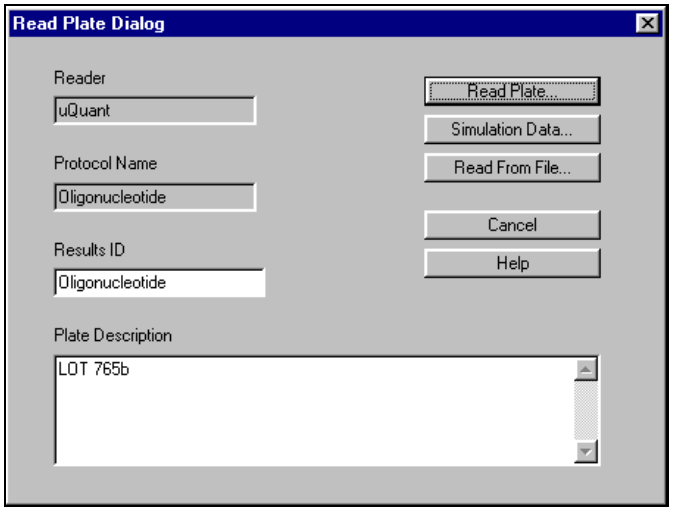
To read a microplate with a protocol:

1. Ensure that the reader is properly attached to the computer, and that the correct reader type and communication parameters are selected under **Setup|Reader.**
2. Open an existing protocol or define a new one.

   See either "Opening an Existing Protocol" or "Defining Protocol Parameters" in *Defining Protocols* for more information.

3. Initiate the read by clicking on the **Read Plate** button in the KCjunior main screen, selecting **Protocol|Read Plate,** or by clicking the **toolbar icon.**

   The **Read Plate** dialog appears:

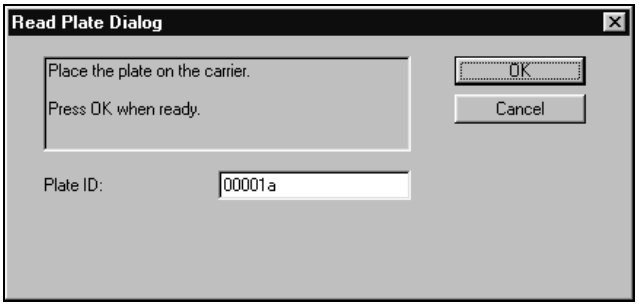


4. Enter a **Results ID,** using up to 32 alphanumeric characters. The ID is optional in this dialog, but it will be required later if the results are to be stored in the Results Database.

   - If performing a **multiple-plate endpoint read,** all of the plates read will be stored in the Results Database under this ID (although they can each have their own Plate ID).

5. Enter a **Plate Description,** using up to 1024 alphanumeric characters. This description is optional.

6. Click the **Read Plate** button. A second **Read Plate** dialog appears.



7. (Optional) Manually assign a **Plate ID** to this plate.
   - For **multiple plate endpoint absorbance protocols,** each individual plate can have its own Plate ID assigned. This prompt appears before each plate is read.
8. Place the plate on the reader, then click **OK** to begin the plate read.
   - If the attached reader is equipped with a barcode scanner *and* **Use Reader's Barcode Scanner for Plate ID** is enabled in KCjunior under Setup|Configuration|General, KCjunior attempts to scan the plate. If no barcode is detected, or if the attached reader is not equipped with a barcode scanner, the Plate ID can be entered manually.
   - Click the **Abort Read** button to halt the read in progress. Data reduction is based on any data retrieved up to the time the button was clicked.
   - When the plate read is complete, KCjunior automatically retrieves the measurements from the reader, performs data reduction, and displays the results. See *Viewing Results* or *Analyzing and Manipulating Results* for more information.
9. To **save** the results:
   - Select **Results|Save Results,** or press **CTRL+S.**

     KCjunior requests a **Results ID** if one was not defined before the plate read. Enter the ID using up to 32 alphanumeric characters. This is the name under which the record will be stored in the Results Database.

     Click **OK** to save the results.

Simulating a Plate Read

Note: *Simulation Data is not supported for the* ***spectral scan*** *read method.*

KCjunior provides the option to **simulate** a plate read by entering plate data manually, or by copying data from a spreadsheet and pasting it into a KCjunior plate. Simulation data typically represents previously acquired measurements entered into KCjunior for analysis.

Simulation data can be generated with or without a protocol open, and it can be saved, accessed, and modified. To simulate a plate read:

1. Ensure that under **Setup|Reader,** the desired **Reader Type** is currently selected. The reader does not need to be connected to the computer to simulate a plate read.
2. If desired, open an existing protocol or define a new one.
3. Initiate the simulation by clicking the **Read Plate** button in the KCjunior main screen, selecting **Results|Read Plate,** or by clicking the **toolbar icon.**

   The **Read Plate** dialog appears.

4. Enter a **Results ID,** using up to 32 alphanumeric characters. The ID is optional in this dialog, but it will be required later if the results are to be stored in the Results Database.
   - If performing a **multiple-plate endpoint read,** all of the plates in the read will be stored in the Results Database under this Results ID.
5. Enter a **Plate Description,** using up to 1024 alphanumeric characters. This description is optional.
6. Click the **Simulation Data** button.

   Note: If there is no protocol open, the **Read Method** dialog appears. Enter the basic Read Method parameters, then click **OK** to continue.

The **Simulation Data** dialog appears, in a format corresponding with the defined Plate Geometry.

7. Enter OD, RFU, or RLU values directly into the appropriate "wells", or copy values from the spreadsheet and paste them here by pressing **Ctrl+V.**
   - If a **dual-wavelength** Endpoint or Kinetic absorbance read is defined, the value entered in each well must represent the *delta OD* for that well.
   - Set the **Kinetic Read** value to simulate each plate reading in the kinetic reading cycle.
   - Set the Wavelength value to simulate each wavelength reading in a **MultiWavelength** read.
8. When the data entry is complete, click **OK.** KCjunior performs data reduction based on the defined protocol parameters, if applicable, and then displays the results.

Reading Plate Data from a File

KCjunior can read plates that were generated with Bio-Tek's **KCJr for DOS** software, and absorbance files generated by Bio-Tek's **OMNI System.**

To read plate data from one of these files:

1. If desired, create a protocol or open an existing one. The read method parameters should be appropriate for the file to be opened.

   Note: Before creating or defining a protocol to accommodate a multi-wavelength Omni-System absorbance file, make sure the reader type specified under **Setup|Reader** supports multi-wavelength reads. The reader does not need to be connected to the computer to read plate data from a file.

2. Select **Results|Read Plate,** or click the **Read Plate** button in the main screen.

   The **Read Plate** dialog appears.

3. Enter a **Results ID,** using up to 32 alphanumeric characters. The ID is optional in this dialog, but it will be required later if the results are to be stored in the Results Database.

4. (Optional) Enter a **Plate Description,** using up to 1024 alphanumeric characters.

5. Click the **Read From File** button. A standard **Open File** dialog appears.

6. Locate and highlight the desired file, then click **Open.**

   KCjunior examines the file to determine certain characteristics such as read method, wavelengths, number of reads, and kinetic interval (if applicable). If there are no discrepancies, KCjunior opens the file and calculates the results.

   If KCjunior is unable to determine one or more of these characteristics, a **Read File** dialog appears, requesting entry of the unknown values.

   The **Read File** dialog contains as much information as KCjunior was able to retrieve from the file. Any unknown values must be entered before results calculation can begin.

   - If a field is dimmed and inaccessible, KCjunior requires no further input.
   - If the **Read Method** field drop-down box is accessible, choose **Endpoint** or **Kinetic** as applicable.
   - If the **Primary Wavelength** field contains 0, enter a wavelength from **1** to **999.**

- If the **Reference Wavelength** field contains 0, enter a wavelength from **1** to **999,** or leave it set to 0 if no reference wavelength is required.
- If the **Kinetic Interval** field is accessible, enter a value (in seconds) from **1** to **9999.**
- Click **OK** to accept the values. KCjunior validates the settings and then calculates the results.

7. To **save** the results, select **Results|Save Results,** or press **CTRL+S.**
8. KCjunior requests a **Results ID** if one was not defined before the plate read. Enter the ID using up to 32 alphanumeric characters. This is the name under which results will be stored in the Results Database.
9. Select **OK** to save the results.

Collecting Data from an EL301 Microstrip Reader

The **EL301 microstrip reader** does not support computer control for its operation. Optical density data stored in the reader, however, can be transferred to KCjunior for analysis.

- KCjunior accepts **EL301** data in **Absorbance (ABSORB)** mode, and does not accept data in the Ratio (RATIO), Linear Equation (LINEQU), or Difference (DIFF) modes.
- KCjunior accepts data for up to **96 wells** with each transfer.
  - To transfer two or three **plates** of data to KCjunior, send them separately and save each plate under a different name.
- If a blank well was read, the EL301 automatically performs **blank subtraction** before data transfer.
- If a dual-wavelength read is performed, the EL301 automatically calculates the **Delta OD** before data transfer.

To collect data from an **EL301 reader:**

1. Perform the necessary microstrip reads.
2. Connect the reader to the computer on which KCjunior is loaded.
   - Consult the EL301 Operator's Manual for instructions on "Connecting the Model EL301 to an External Computer".

3. In KCjunior, define the **Reader Setup** parameters as follows:

   Reader Type: EL301

   Com Port: Communication port on computer

   Baud Rate: 1200

   Data Bits: 8

   Parity: None

   Stop Bits: 2

   EOT Character: 4

4. Open an existing or define a new **protocol.**

   - The Well ID Template should correspond with the type of strip(s) read.

     8-well strips: A1 to H1, A2 to H2, … A12 to H12.

     12-well strips: A1 to A12, B1 to B12, … F1 to F12.

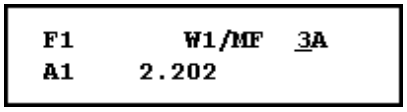
     16-well strips: A1 to H2, A3 to H4, … A11 to H12.

5. Select **Results|Read Plate,** or click the **Read Plate** button.

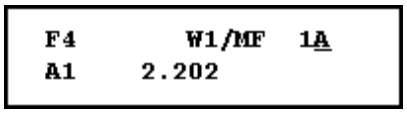
   KCjunior displays the prompt "Press DATA OUT key on reader when ready".

6. Within the reader's main menu, select the data to be transferred, then press the **DATA OUT** key. The optical density values are transferred to KCjunior.

   - On the EL301 display, make sure the cursor is positioned under the **Strip Number** to send data for one strip, or under the **Plate Letter** to send data for up to six strips.

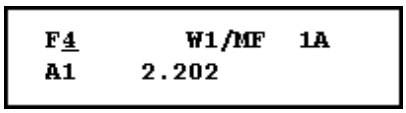


     The picture above shows the cursor positioned under the **Strip Number.** Data for File 1, Plate A, Strip 3 will be sent to KCjunior.



     The picture above shows the cursor positioned under the **Plate Letter.** Data for File 4, Plate A, Strips 1 to 6 will be sent to KCjunior.

- Do **not** position the cursor under the **File Number,** as shown below:



- Consult the EL301 Operator's Manual for more information on "Data Output to Peripheral Devices".

7. Data retrieved from the EL301 can be analyzed, manipulated, saved, and printed in the same manner as any other set of plate data in KCjunior.
   - Overrange reads, unread strips, and unread wells appear as ****** in KCjunior.

Reader Runtime Errors

If the reader experiences an error, KCjunior attempts to perform data reduction with any data that it received prior to the failure.

For example, if the reader experiences an error during the 10th of 12 kinetic reads, results are generated based on reads 1 through 9. KCjunior also displays appropriate error messages with the results.

- Refer to the *reader's* user guide for information on error messages displayed on the reader.
- See **Communication Errors** in the *Troubleshooting* section for more information on KCjunior's error and warning messages.

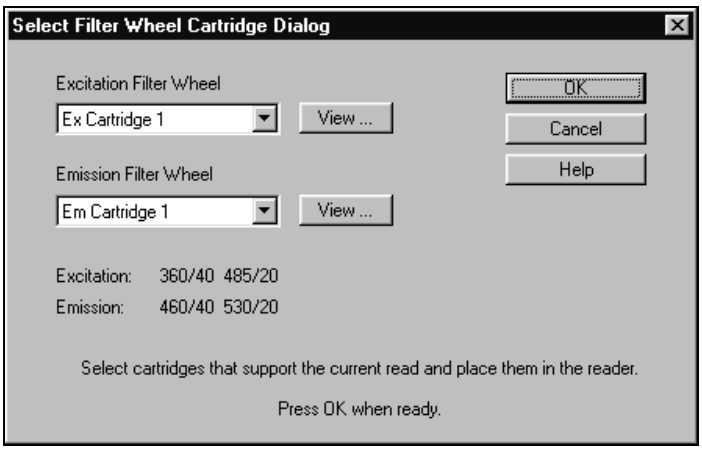
Comments/Warnings

Following data reduction, if an error is detected or a condition is present that requires notification, a **Comments / Warnings** button becomes available in the results display. Click this button to see the comments and/or warnings generated for the plate.

- See **Comments and Warnings** in the *Troubleshooting* section for specific information on data reduction comments and warnings.

Selecting a Filter Wheel Cartridge

Depending on the prompt options settings in the Setup|Configuration| Filter Wheel Configuration dialog, the **Select Filter Wheel Cartridge** dialog may appear (applies to FLx800 reader only).



This dialog shows the Excitation and Emission filters required by the protocol. The selected filter wheel cartridges must contain filters to support the protocol.

1. Using the drop-down menus, **select** the desired Excitation and Emission Filter Wheel cartridges.
   - The contents of Excitation and Emission filter wheel cartridges are defined under **Setup|Configuration.**
2. After selecting a cartridge, clicking **View** displays the cartridge's contents.
   - Click **Cancel** to return to the Select Filter Wheel Cartridge dialog.
3. Click **OK** to save the changes.
   - If the selected cartridge(s) do not contain the necessary filters to support the current protocol, either select a different cartridge or modify the protocol.

Exporting Data

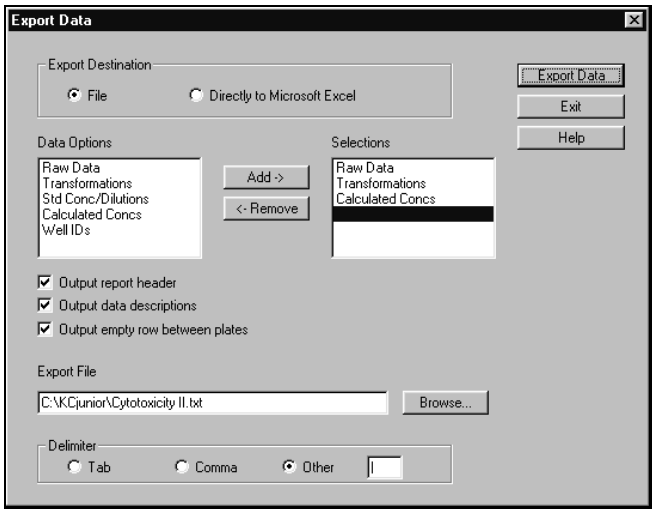
Plate data can be exported to a **file** or directly into a **Microsoft Excel spreadsheet.**

The data options available for export depend on the current protocol parameters. For example, if the protocol performs blanking, uses transformations, and generates a standard curve, the raw data, blanked data, transformation results, and/or calculated concentrations can be exported.

Note: If no protocol is open, the **raw data** can still be exported.

To **export data** to a file or to an Excel spreadsheet:

1. Open/Create a protocol (if desired), then read/open a plate.
2. Select **Results|Export Data.** The **Export Data** dialog appears:



   - Review the items under "Essential Exporting Information" on page 6-23 before performing a data export.

3. From within the Export Data dialog, choose an **Export Destination.**
   - Choose **File** to export the data to a text file. The **Export File** and **Delimiter** options are presented. See "Exporting Data to a File" on page 6-19.
   - Choose **Directly to Microsoft Excel** to export the file to an Excel spreadsheet. The **Start Excel Now** button and **First Excel Cell Location** option are presented. See "Exporting Data Directly to Microsoft Excel" on page 6-21.

Exporting Data to a File

To export data to a file:

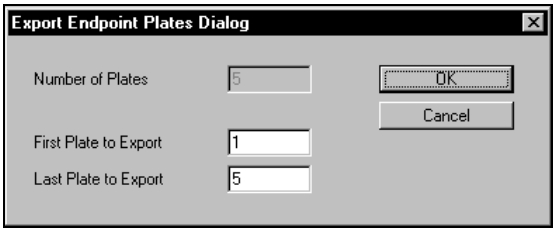
*Select **Results|Export Data** to open the Export Data dialog.*

1. From within the Export Data dialog, choose the Export Destination option **File.**
2. **Select the Data Option(s)** to be exported.
   - In the Data Options box, highlight the item(s) to be exported, then click the **Add** button. The selected item(s) will appear in the **Selections** box.
   - To **remove** an item from the Selections box, highlight the item and click the **Remove** button (or double-click).
   - To **insert** a data option between two items in the Selection box, highlight the item in the Selection box above which the new item will be inserted, highlight the item in the Data Options box, then click **Add.**
3. Check **Output report header** to include the Plate ID and the date and time of the plate reading.
4. Check **Output data descriptions** to include identifying information for each set of plate data.
   - See "Sample Output with Report Header and Data Descriptions" on page 6-23.
5. Check **Output empty row between plates** to add a blank line after each set of plate data.
   - See "Sample Output with Empty Row Between Plates" on page 6-23.
6. Enter a valid path and file name in the **Export File** field.
   - Example: C:\KCjunior\Results\Export 01.txt
   - Long file names are supported. If no file extension is specified, KCjunior automatically uses .txt (text file).
   - Alternatively, click **Browse** to search the directory structure. Locate and highlight the desired file name, then click **Open** to select it, or travel to the desired file storage location, enter a new file name, then click **Open** to create/select it.
   - The file can be **stored** on a computer's hard drive, on a network to which the computer is attached, or on a floppy disk.
   - If the file name already exists, KCjunior provides the option to **overwrite** the entire contents of the file or to **append** the new data to the end of the file.

7. Choose a **Delimiter,** or define a new one.
   - KCjunior uses **delimiters** to separate individual values such as raw ODs, calculated concentrations, and transformation results.
   - Choose **Tab** or **Comma,** or choose **Other** and enter a custom delimiter, such as | or #.

     Example: If the **comma** delimiter is used, exported Raw Data will resemble the following:

     ```
     1.235,0.267,0.541,1.749,0.232,2.418
     0.784,0.154,1.982,0.247,1.583,3.148
     2.961,0.235,1.287,1.768,0.336,0.234
     0.984,2.514,1.674,3.992,1.852,0.235
     ```
   - **Tip:** If the file will be **imported** by another application, check to see which delimiter(s) that application accepts.
8. Click the **Export** button.
   - If the file name already exists, KCjunior provides the option to **overwrite** the entire contents of the file or to **append** the new data to the end of the file.
   - **Note:** If exporting data for a multiple-plate endpoint read, the **Export Endpoint Plates** dialog appears.

     Specify the **First** and **Last plates** to export, then click **OK** to continue.

     Export format: All selected data sets are exported for the first plate, then for the second plate, and so on through the last plate.

Exporting Data Directly to Microsoft Excel

To export data to Microsoft Excel:

*Select **Results|Export Data** to open the Export Data dialog.*

1. From within the Export Data dialog, choose the Export Destination option **Directly to Microsoft Excel.**
2. If exporting to an **existing** Excel spreadsheet:
   - Click **Start Excel now** to launch Excel immediately.
   - Open the spreadsheet.
   - Review the spreadsheet to determine what data is expected and where it should be stored.
   - **Important!** If the spreadsheet contains formulas or other important information, take the following into consideration to avoid overwriting these cells, and to ensure that the data exports to the correct cells:

     'well' is the KCjunior term referring to a location in the microplate, and **'cell'** is the Microsoft Excel term referring to a location in the spreadsheet.

     The spreadsheet may contain formulas that reference other cells in the spreadsheet. Be sure to define the export parameters so that the correct set of plate data is sent to the correct cells.

     KCjunior exports data starting with well A1 in the specified **First Excel Cell Location.**
   - Switch back to KCjunior.
3. **Select the Data Option(s)** to be exported.

After each export, KCjunior retains the list of Selections (up to 15), for use with the next export. This is particularly useful if exporting the same set(s) of plate data for multiple plates.

   - In the Data Options box, highlight the item(s) to be exported, then click the **Add** button. The selected item(s) will appear in the **Selections** box.
   - To **remove** an item from the Selections box, highlight the item and click the **Remove** button (or double-click).
   - To **insert** a data option between two items in the Selection box, highlight the item in the Selection box above which the new item will be inserted, highlight the item in the Data Options box, then click **Add.**
4. Check **Output report header** to include the Plate ID and the date and time of the plate reading.
5. Check **Output data descriptions** to include identifying information for each set of plate data.
   - See "Sample Output with Report Header and Data Descriptions" on page 6-23.

6. Check **Output empty row between plates** to add an empty row after each set of plate data.

   ➢ See "Sample Output with Empty Row Between Plates" on page 6-23.

7. Specify the location (ex. A1, C3, K15) of the **first cell** in the current Microsoft Excel spreadsheet.

   ➢ This is where the first entry will be made in the spreadsheet.

   If **Output Report Header** is enabled, the header is stored in this location, followed by a blank row. The first data value (such as the contents of well A1), is stored below the specified "first cell". Subsequent values then fill in to the right of and below the first data value.

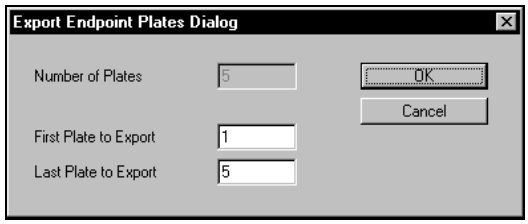
   ➢ See the sample Excel spreadsheet after data export below:

	A	B	C	D	E	F	G
1							
2		Plate: 1739A Date Created: 11/30/2000 10:37:16 AM					
3							
4		Raw Data					
5		0.000	0.500	0.667	0.750	0.800	0.833
6		1.423	1.429	1.433	1.438	1.441	1.444
7		1.992	1.992	1.993	1.993	1.993	1.993
8		2.028	2.029	2.031	2.032	2.033	2.035

   ➢ **Tip:** If performing multiple exports to the same spreadsheet, use the First Excel Cell Location and Output empty row between plates features to **append** one data set to another, with a row between each set.

8. Click the **Export** button.

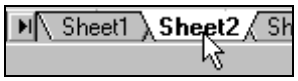
   ➢ **Note:** If exporting data for a multi-plate endpoint read, the **Export Endpoint Plates** dialog appears.



   Specify the **First** and **Last plates** to export, then click **OK** to continue.

   Export format: All selected data sets are exported for the first plate, then for the second plate, and so on through the last plate.

   ➢ KCjunior launches Excel if it is not already open and then exports the data to the current Sheet.



Tip: To avoid overwriting the contents of "Sheet1" in Microsoft Excel, choose another Sheet before performing the next data export.

Essential Exporting Information

- Certain numeric data is **annotated** by KCjunior when it is displayed. Numeric data is exported, however, *without* these annotations.

  Examples of annotations include: Masked wells contain a strike-through, extrapolated concentrations are surrounded by ( ), and ambiguous concentrations are surrounded by { }.

- Data that is currently a **string,** such as OUT, *****, or ?????, is exported exactly as it is displayed in KCjunior.

- Data for **all wells** on the plate is exported, even if the well is considered to be "empty".

  For all data options, a well is empty if no ID is assigned to it. For some data options (such as Blanked Data), a well is empty if its Raw Data value is currently masked.

- The export utility is not available for the **spectral scan** read method.

Sample Output with Report Header and Data Descriptions

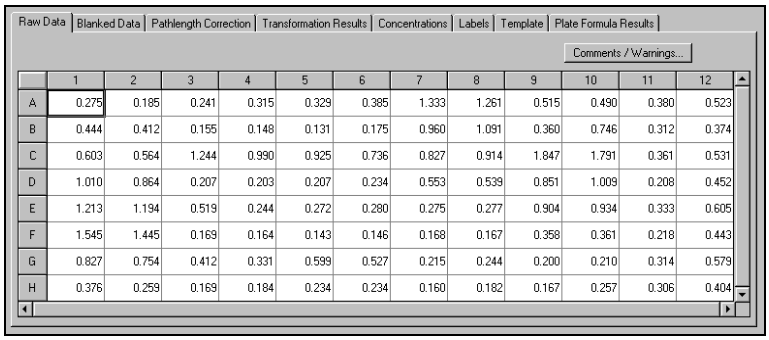
```
Raw Data (405)

0.000,0.500,0.667,0.750,0.800,0.833,0.857,0.875,0.889,0.900
1.423,1.429,1.433,1.438,1.441,1.444,1.447,1.450,1.990,1.991
1.992,1.992,1.993,1.993,1.993,1.993,2.017,2.019,2.021,2.022
2.028,2.029,2.031,2.032,2.033,2.035,2.036,2.037,2.038,2.039
```

Sample Output with Empty Row Between Plates

```
Kinetic Read 1 (00:00:00)

0.000,0.500,0.667,0.750,0.800,0.833,0.857,0.875
1.423,1.429,1.435,1.438,1.421,1.434,1.497,1.450
1.992,1.992,1.993,1.993,1.993,1.993,2.017,2.019
2.028,2.029,2.031,2.032,2.033,2.035,2.036,2.037

       (empty row here)

Kinetic Read 2 (00:10:00)

1.010,1.465,1.557,1.793,1.510,1.835,1.987,1.675
2.423,2.429,2.433,2.438,2.441,2.484,2.447,2.450
2.982,2.942,2.895,2.045,2.963,2.223,3.017,3.019
3.028,3.029,3.031,3.032,3.033,3.035,3.036,3.037
```

Viewing Results

When plate reading is complete, or after simulation data has been entered or data has been read from a file, KCjunior displays the results in a format similar to the one shown below:



	1	2	3	4	5	6	7	8	9	10	11	12
A	0.275	0.185	0.241	0.315	0.329	0.385	1.333	1.261	0.515	0.490	0.380	0.523
B	0.444	0.412	0.155	0.148	0.131	0.175	0.960	1.091	0.360	0.746	0.312	0.374
C	0.603	0.564	1.244	0.990	0.925	0.736	0.827	0.914	1.847	1.791	0.361	0.531
D	1.010	0.864	0.207	0.203	0.207	0.234	0.553	0.539	0.851	1.009	0.208	0.452
E	1.213	1.194	0.519	0.244	0.272	0.280	0.275	0.277	0.904	0.934	0.333	0.605
F	1.545	1.445	0.169	0.164	0.143	0.146	0.168	0.167	0.358	0.361	0.218	0.443
G	0.827	0.754	0.412	0.331	0.599	0.527	0.215	0.244	0.200	0.210	0.314	0.579
H	0.376	0.259	0.169	0.184	0.234	0.234	0.160	0.182	0.167	0.257	0.306	0.404

Initially, **Raw Data** results are displayed. Depending on the protocol definition, KCjunior may also display **Blanked Data, Pathlength Correction, Transformation Results, Concentrations, Labels, Template,** and **Plate Formula Results.**

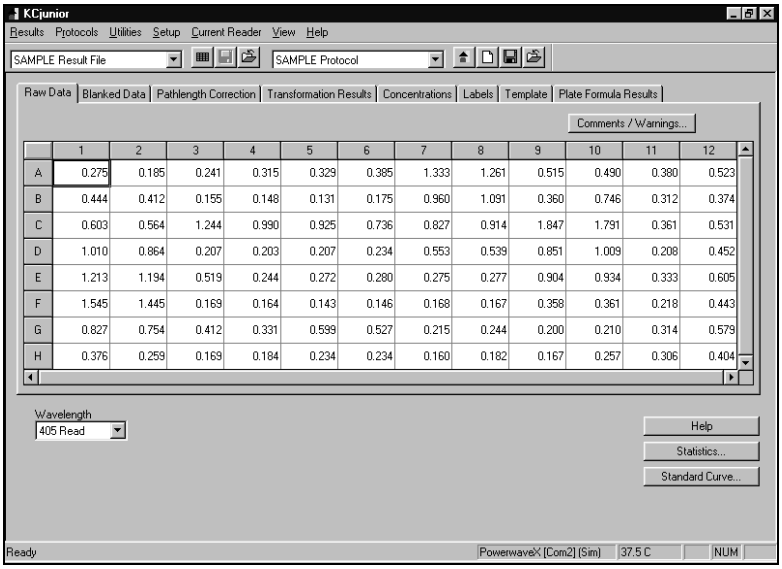
Some "quick tips" are listed here. Detailed information can be found in the next section, *Interpreting Results.*

- To view a particular type of data, click on its "tab".
- To view statistical information for a particular type of data, click on its tab then click the **Statistics** button.
- To "mask" a well (temporarily exclude it from calculations), place the cursor over the desired well, click the right mouse button, then select **Mask Well.**
- To access more detailed information about the plate, place the cursor in the matrix, click the right mouse button, then select **Plate Information.**
- To access more detailed information for a particular well, place the cursor over the desired well, click the right mouse button, then select **Well Information.**
- If a **Comments / Warnings** button is available, click it to see the data reduction comments and/or warnings generated for the plate.
- To export data to a file or directly into Microsoft Excel, select **Results|Export Data.**
- To **copy** the data to another application, highlight the desired wells, then press **CTRL+C.** Switch to the destination application, then press **CTRL+V** (or select a **Paste** option).

Interpreting Results

Overview

When plate reading is complete, or after simulation data has been entered or data has been read from a file, KCjunior displays the **results,** as shown below:



	1	2	3	4	5	6	7	8	9	10	11	12
A	0.275	0.185	0.241	0.315	0.329	0.385	1.333	1.261	0.515	0.490	0.380	0.523
B	0.444	0.412	0.155	0.148	0.131	0.175	0.960	1.091	0.360	0.746	0.312	0.374
C	0.603	0.564	1.244	0.990	0.925	0.736	0.827	0.914	1.847	1.791	0.361	0.531
D	1.010	0.864	0.207	0.203	0.207	0.234	0.553	0.539	0.851	1.009	0.208	0.452
E	1.213	1.194	0.519	0.244	0.272	0.280	0.275	0.277	0.904	0.934	0.333	0.605
F	1.545	1.445	0.169	0.164	0.143	0.146	0.168	0.167	0.358	0.361	0.218	0.443
G	0.827	0.754	0.412	0.331	0.599	0.527	0.215	0.244	0.200	0.210	0.314	0.579
H	0.376	0.259	0.169	0.184	0.234	0.234	0.160	0.182	0.167	0.257	0.306	0.404

The **Raw Data** set of results is always displayed. **Raw Data** represents the measurements that were either retrieved from the microplate reader, entered manually, or read from a file. Results are arranged with rows and columns that align with the specified plate geometry.

Depending on the protocol definition, **Blanked Data, Pathlength Correction, Transformation Results, Concentrations, Labels, Template,** and **Plate Formula Results** may also be displayed.

Interpreting Results begins by describing the **Well Notations** that may appear in one or more results categories, then explains how to interpret each of the eight possible sets of results.

Well Notations

Within the results displays, different circumstances may cause the result in a well to be displayed with a special symbol, such as a ~~strike-through~~ or parenthesis ( ), or to be completely replaced with symbols such as ?????? or OUT.

The following list describes the well notations that may appear in results displays and on reports:

- If a well contains **OUT** in place of an OD, RFU, or RLU, the raw data value fell outside the acceptable min/max data range defined in the protocol, and it has been excluded from further calculations.
  - See "General Information" in *Defining Protocols* for information on setting Raw Data Limits and Blank Limits.
- If an OD, RFU, or RLU value is enclosed in **brackets [2.999],** the raw data value fell outside the acceptable min/max data range defined in the protocol, and was automatically set to the upper or lower limit of this range as appropriate.
- If a Raw data value contains a **strike-through (2.684),** it has been **masked,** or temporarily removed from calculations. See *Results Options* at the end of this section for information on masking and unmasking wells.
- A well containing **DIV/0** indicates a failed attempt to divide by zero.
- A well containing **asterisks (******)** indicates a well status error.
- If the concentration in a well is enclosed in **parenthesis (20.345),** it was calculated through extrapolation.
- If the concentration in a well is enclosed in **curly braces {10.765},** it is ambiguous.
- An **empty** well indicates no result was calculated. Possible reasons include:
  - If a protocol is open, no Well ID was assigned to this location.
  - The Raw Data value is currently masked.
  - The Raw Data value is out of range (OUT).
  - If the well is empty under Concentrations, Extrapolation may be disabled.

- A well containing **question marks (??????)** indicates that a value could not be calculated. Possible reasons include:
  - The well references another well with a status of:

    Well Read Error

    OUT

    Masked

    Divide by Zero

    Well Failure
  - A calculation within or involving the well:

    attempted an invalid logarithmic function.

    is syntactically invalid.

    references an invalid location.

    references an invalid Well ID.

    attempted a Standard Deviation on a well group with an insufficient sample population.

    attempted a Coefficient of Variation on a well group with an insufficient sample population.
  - The well is in a blank sector that contains blanks but none of them are valid.
  - Cutoffs could not be evaluated for the well.
  - A concentration had no valid solutions on the generated curve.
  - The curve could not be generated.

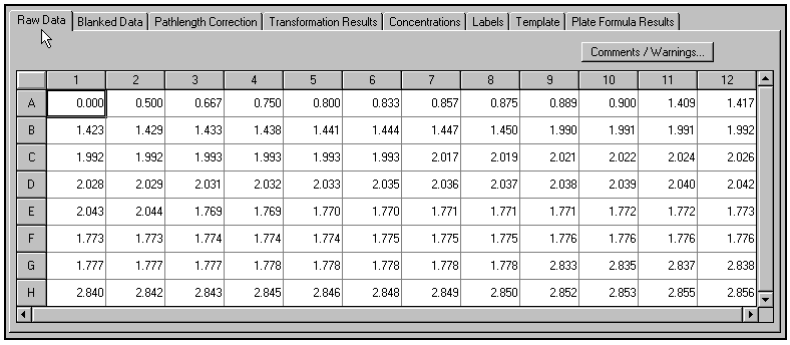
Raw Data

Raw Data represents the original measurements that were either retrieved from the microplate reader, entered manually, or read from a file.

The format of the Raw Data display differs based on the defined **Read Method** (Endpoint, Kinetic, MultiWavelength, or Spectral Scan), as described on the following pages.

Raw Endpoint Read Data

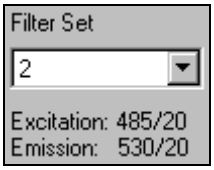
Raw Data results for an **endpoint** read are shown below:



	1	2	3	4	5	6	7	8	9	10	11	12
A	0.000	0.500	0.667	0.750	0.800	0.833	0.857	0.875	0.889	0.900	1.409	1.417
B	1.423	1.429	1.433	1.438	1.441	1.444	1.447	1.450	1.990	1.991	1.991	1.992
C	1.992	1.992	1.993	1.993	1.993	1.993	2.017	2.019	2.021	2.022	2.024	2.026
D	2.028	2.029	2.031	2.032	2.033	2.035	2.036	2.037	2.038	2.039	2.040	2.042
E	2.043	2.044	1.769	1.769	1.770	1.770	1.771	1.771	1.771	1.772	1.772	1.773
F	1.773	1.773	1.774	1.774	1.774	1.775	1.775	1.775	1.776	1.776	1.776	1.776
G	1.777	1.777	1.777	1.778	1.778	1.778	1.778	1.778	2.833	2.835	2.837	2.838
H	2.840	2.842	2.843	2.845	2.846	2.848	2.849	2.850	2.852	2.853	2.855	2.856

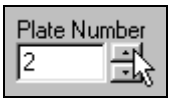
- For **single-wavelength absorbance** reads, each well contains the OD measured at the **Primary** wavelength.
- For **dual-wavelength absorbance** reads, each well contains the *delta OD* value calculated by the reader.

  The reader calculates the delta OD by subtracting the measurement at the Reference wavelength from the measurement at the Primary wavelength.



- For **fluorescence** and **luminescence** reads, 1 to 6 sets of raw data are presented, depending on the number of filter sets specified in the protocol.

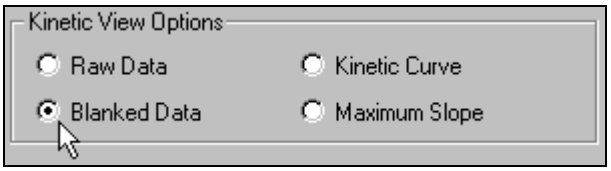
  Initially, the RFU/RLU values for filter set #1 are presented. Use the drop-down menu at the bottom of the screen to choose a different filter set.



- For **multi-plate endpoint** reads, use the Plate Number selection box to choose a different plate.

Raw and Blanked Kinetic Read Data

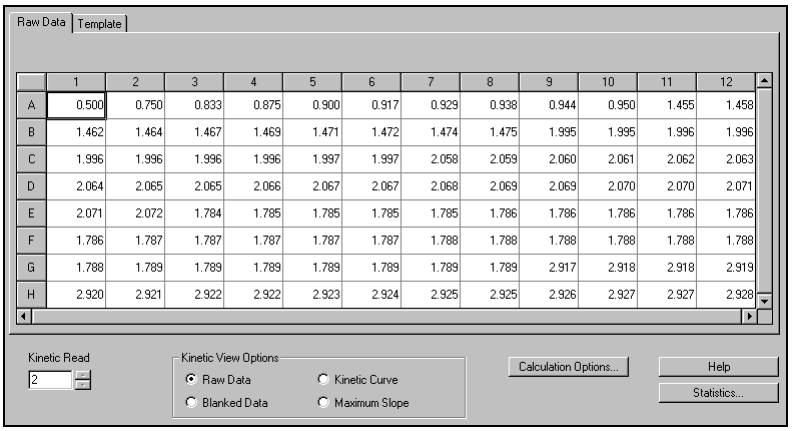
Raw *and* blanked kinetic data are viewed under the Raw Data tab. View options include sets of individual kinetic reads, the kinetic curve for each well, and the maximum slope for each well.



To switch between the different views, click the appropriate **Kinetic View Options** radio button.

Individual Kinetic Reads

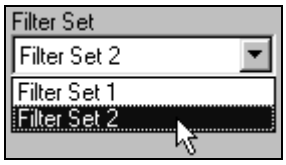
Raw Data results for individual kinetic reads are shown below:



	1	2	3	4	5	6	7	8	9	10	11	12
A	0.500	0.750	0.833	0.875	0.900	0.917	0.929	0.938	0.944	0.950	1.455	1.458
B	1.462	1.464	1.467	1.469	1.471	1.472	1.474	1.475	1.995	1.995	1.996	1.996
C	1.996	1.996	1.996	1.996	1.997	1.997	2.058	2.059	2.060	2.061	2.062	2.063
D	2.064	2.065	2.065	2.066	2.067	2.067	2.068	2.069	2.069	2.070	2.070	2.071
E	2.071	2.072	1.784	1.785	1.785	1.785	1.785	1.786	1.786	1.786	1.786	1.786
F	1.786	1.787	1.787	1.787	1.787	1.787	1.788	1.788	1.788	1.788	1.788	1.788
G	1.788	1.789	1.789	1.789	1.789	1.789	1.789	1.789	2.917	2.918	2.918	2.919
H	2.920	2.921	2.922	2.922	2.923	2.924	2.925	2.925	2.926	2.927	2.927	2.928

Kinetic data is initially displayed in this format, reporting the raw values for each well of **Kinetic Read #1.**

- For **single-wavelength absorbance** reads, each well contains the OD measured at the **Primary** wavelength.
- For **dual-wavelength absorbance** reads, each well contains the *delta OD* value calculated by the reader. The reader calculates the delta OD by subtracting the measurement at the Reference wavelength from the measurement at the Primary wavelength.
- For **fluorescence** or **luminescence** reads, each well contains the RFU or RLU value calculated for the the *first* filter set defined in the protocol. If *two* filter sets are defined, use the Filter Set drop-down menu to choose a different filter set.



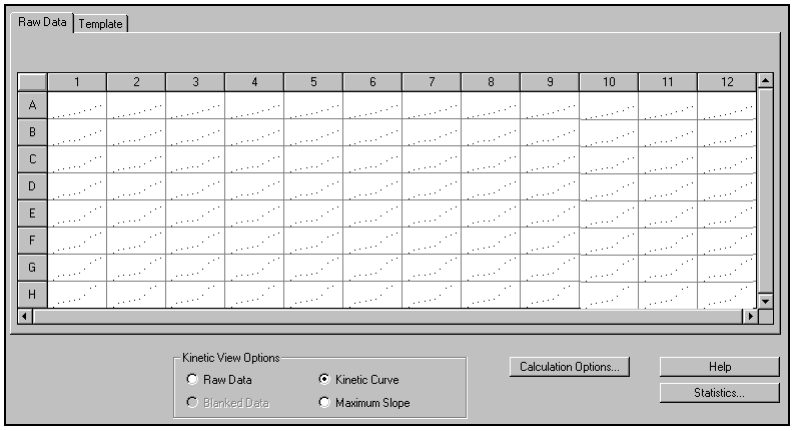
- If blank wells were defined in the Well ID template, click the **Blanked Data** radio button to view the data after blank subtraction.
- To view the results for a different kinetic read, enter the desired read number in the **Kinetic Read** field (or click the up/down arrow buttons).
- Click the **Calculation Options** button to adjust the kinetic read limits, to alter the maximum slope calculation, or to choose a different maximum slope unit of measure.

Kinetic Curves

Kinetic read results are shown below, in the **Kinetic Curve** format:

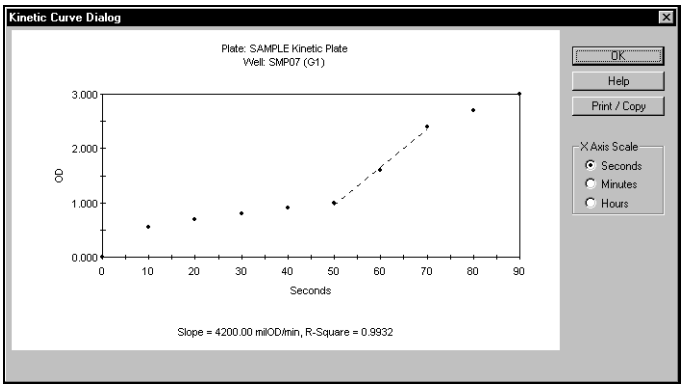
*To **print** the Kinetic Curves display as shown here:*

Press Alt+Print Screen to copy the screen shot to the Clipboard, paste the Clipboard contents into (for example) an empty word processing document, then print the document.



- In this format, each well initially displays a graph of the data points corresponding to the OD, RFU, or RLU readings taken over time.
- If blank subtraction was performed, the kinetic curves are automatically based on the **Blanked** values.
- Click the **Calculation Options** button to adjust the kinetic read limits, to alter the maximum slope calculation, or to choose a different maximum slope unit of measure.
- To **enlarge** the view of the curve in an individual well, place the cursor over the desired well and click the left mouse button.

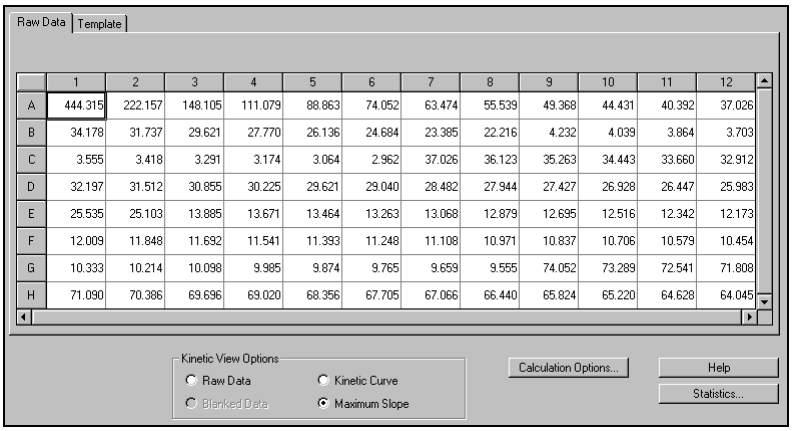
  The **Kinetic Curve** dialog appears:



- The **Kinetic Curve** and its **Maximum Slope** and **R-Square** values are displayed for the well.
- Click **OK** to return to the **Kinetic Curve** results display.

Maximum Slope

Kinetic read results are shown below, in the **Maximum Slope** format:



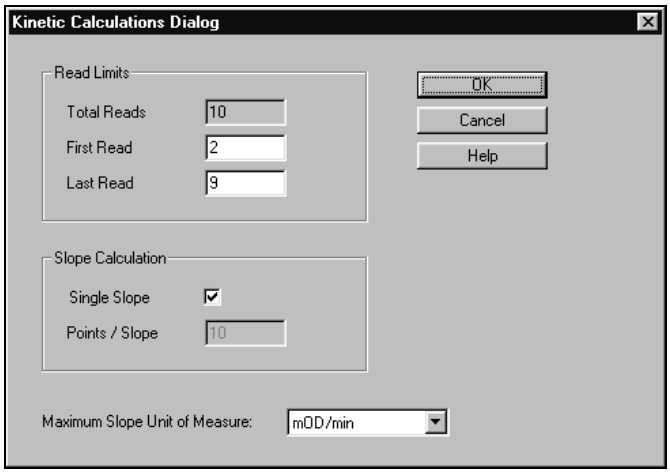
	1	2	3	4	5	6	7	8	9	10	11	12
A	444.315	222.157	148.105	111.079	88.863	74.052	63.474	55.539	49.368	44.431	40.392	37.026
B	34.178	31.737	29.621	27.770	26.136	24.684	23.385	22.216	4.232	4.039	3.864	3.703
C	3.555	3.418	3.291	3.174	3.064	2.962	37.026	36.123	35.263	34.443	33.660	32.912
D	32.197	31.512	30.855	30.225	29.621	29.040	28.482	27.944	27.427	26.928	26.447	25.983
E	25.535	25.103	13.885	13.671	13.464	13.263	13.068	12.879	12.695	12.516	12.342	12.173
F	12.009	11.848	11.692	11.541	11.393	11.248	11.108	10.971	10.837	10.706	10.579	10.454
G	10.333	10.214	10.098	9.985	9.874	9.765	9.659	9.555	74.052	73.289	72.541	71.808
H	71.090	70.386	69.696	69.020	68.356	67.705	67.066	66.440	65.824	65.220	64.628	64.045

- The **Maximum Slope** is initially based on the total number of kinetic points ("single slope").
- If blank subtraction is performed, the maximum slope is automatically based on the **Blanked** values.
- Click the **Calculation Options** button to adjust the kinetic read limits, to alter the maximum slope calculation, or to choose a different maximum slope unit of measure.

Adjusting Kinetic Read Limits

Kinetic calculations initially include **all** measurements taken over the duration of the plate read. Kinetic read limits can be adjusted so that calculations are based on a subset of the total number of measurements.

1. From within any kinetic results display, click the **Calculation Options** button to open the **Kinetic Calculations** dialog:



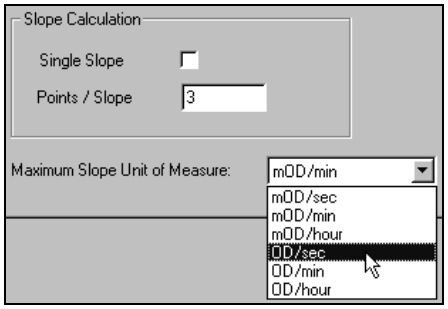
2. To change the number of points for inclusion in the kinetic calculations, enter a new **First Read** and/or **Last Read** value, then click **OK.**

   The kinetic results redisplay, showing only the points in the new calculation range. The **Maximum Slope** automatically recalculates, based on the new calculation range.

Altering the Maximum Slope Calculation

The Maximum Slope is initially calculated based on the **total** number of kinetic points ("single slope"). The number of successive points for inclusion in the slope calculation can be adjusted:

1. From within any kinetic results display, click the **Calculation Options** button to open the **Kinetic Calculations** dialog:

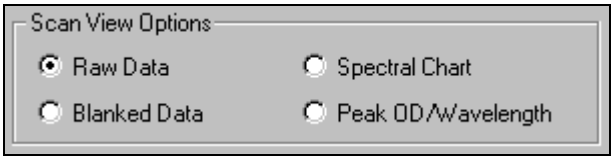


2. Deselect **Single Slope.**
3. Enter a new value in the **Points / Slope** field.
4. (Optional) Select a **Maximum Slope Unit of Measure.** The default is mOD/min.
5. Click **OK.** The kinetic results redisplay. The Maximum Slope automatically recalculates, based on the new Points/Slope value.

Raw and Blanked Spectral Data

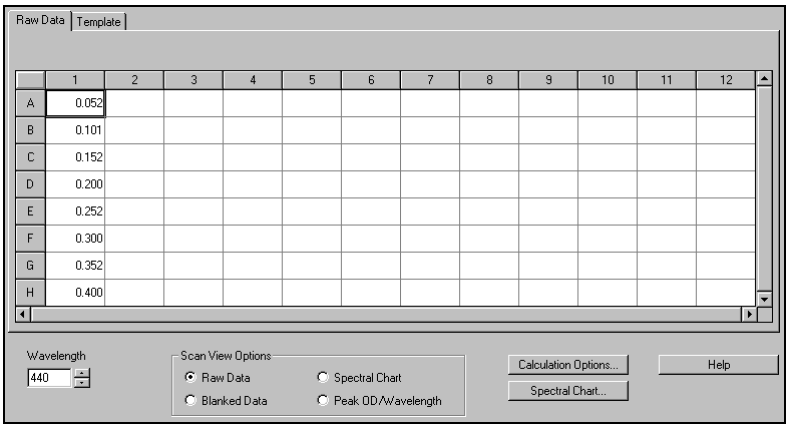
Raw *and* blanked spectral scan data are viewed under the Raw Data tab. View options include sets of ODs at each wavelength, the spectral curve for each well, and the peak OD and corresponding wavelength for each well.

To switch between the different views, click the appropriate **Scan View Options** radio button.



Individual Spectral Reads

Raw Data results for individual spectral reads are shown below:



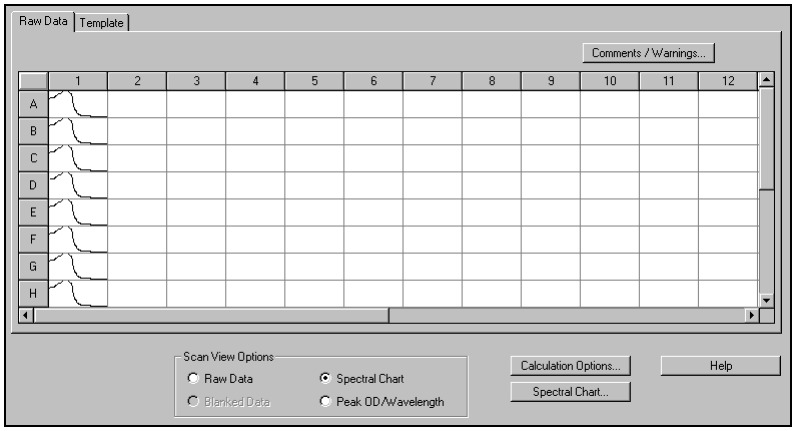
Spectral data is initially displayed in this format, reporting the Raw OD read for each well at the **First Scan Wavelength.**

- To view the Raw ODs for a different wavelength, enter the desired wavelength value in the **Wavelength** field (or use the up/down arrow buttons).
- If a blank well was defined in the Well ID template, click the **Blanked Data** radio button to view the ODs after blank subtraction.

- Click the **Calculation Options** button to adjust the wavelength range used for calculations for all scanned wells.
- Click the **Spectral Chart** button to select up to 8 spectral curves to overlay in one graph.

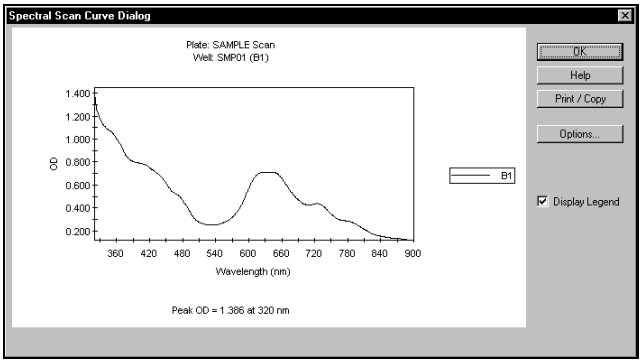
Spectral Chart

Spectral scan results are shown below, in the **Spectral Chart** format:



- In this format, each well displays a graph of the data points corresponding to the OD readings taken across a wavelength range.
- To **enlarge** the view of the curve in an individual well, place the cursor over the desired well and click the **left** mouse button.
- If blank subtraction was performed, the spectral scan curves are automatically based on the **Blanked ODs.**
- Click the **Calculation Options** button to adjust the wavelength range used for calculations for all scanned wells.
- Click the **Spectral Chart** button to select up to 8 spectral curves to overlay in one graph.
- To **enlarge** the view of the curve in an individual well, place the cursor over the desired well and click the left mouse button.

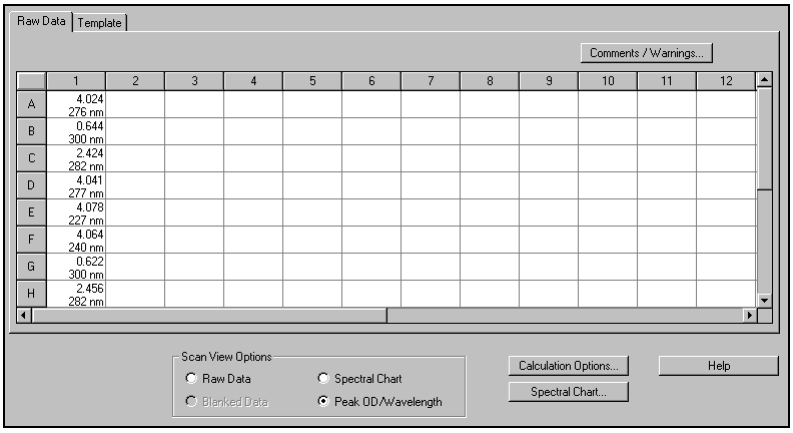
  The **Spectral Scan Curve** dialog will appear:



- The **Spectral Scan Curve** initially contains all ODs read at all wavelengths as defined in the protocol.
- The **Peak OD** and its corresponding wavelength display at the bottom of the graph.
- Move the mouse cursor over a **data point** to see its exact OD and Wavelength values.
- Click **Print/Copy** to print the graph or to copy it to the clipboard.
- Click **Options** to adjust the curve calculations and/or the display options for this well.

Peak OD/Wavelength

Typical spectral scan results are shown below, in the **Peak OD/Wavelength** format:



- The peak (highest) optical density reading and its corresponding wavelength value are reported for each well.
- The **Peak OD** is initially based on the complete wavelength range as defined in the protocol. If the wavelength range is adjusted, the Peak OD is redetermined based on the new range.

- If blank subtraction was performed, the Peak OD is automatically based on the **Blanked ODs.**
- Click the **Calculation Options** button to adjust the wavelength range used for calculations for all scanned wells.
- Click the **Spectral Chart** button to select up to 8 spectral curves to overlay in one graph.

Adjusting the Wavelength Range Used for Calculations

Spectral scan calculations initially include **all** measurements taken across the wavelength range as defined in the protocol. The wavelength range can be adjusted so that calculations are based on a subset of the full wavelength range.

1. From within the **Spectral Scan** results display, click the **Calculation Options** button to open the **Spectral Scan Calculations** dialog.
2. To change the range of wavelengths for inclusion in the spectral scan curve for all scanned wells, enter a new **First Wavelength** and/or **Last Wavelength** value, then click **OK.**

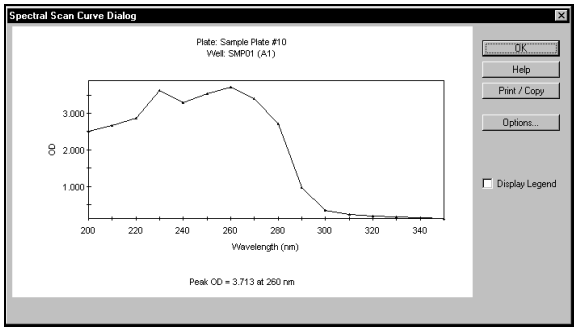
   The Spectral Scan results will redisplay. The spectral curves will show only the points within the new wavelength range, and the Peak ODs will be recalculated.

Curve Display Options

Click the **Calculations Options** button to change the curve display.

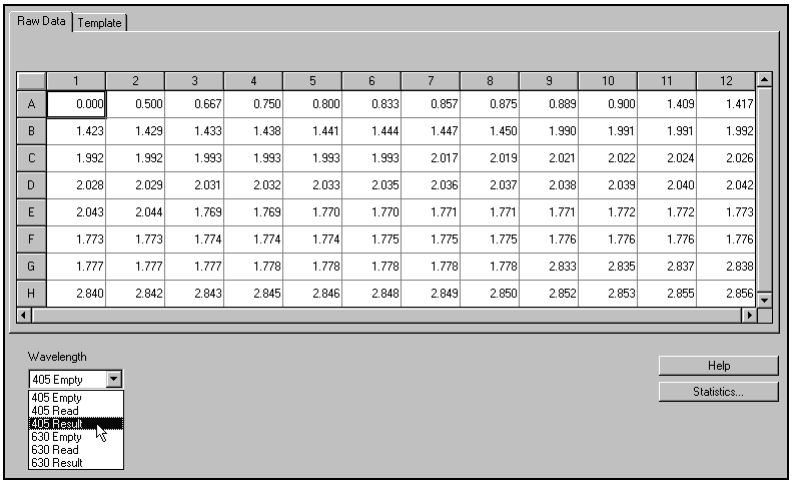
The available curve display options include:

- Display **Points** only.
- Display **Line** only. This is a point-to-point line, shown without the individual data points.
- Display Points **and** Line. This is a point-to-point line, and the data points are displayed. See the example below:



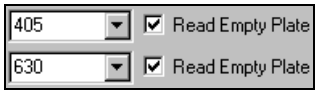
Raw MultiWavelength Read Data

Raw Data results for a **MultiWavelength** read are shown below:



- Each well contains the OD value measured at the wavelength displayed in the **Wavelength** field. Use the drop-down list to choose a different wavelength.
- If an empty plate was read at a particular wavelength, results will be provided for the **Empty** plate, the **Read** plate, and the difference between the two **(Result).**

  The read method parameters shown to the left correspond with the results shown above.



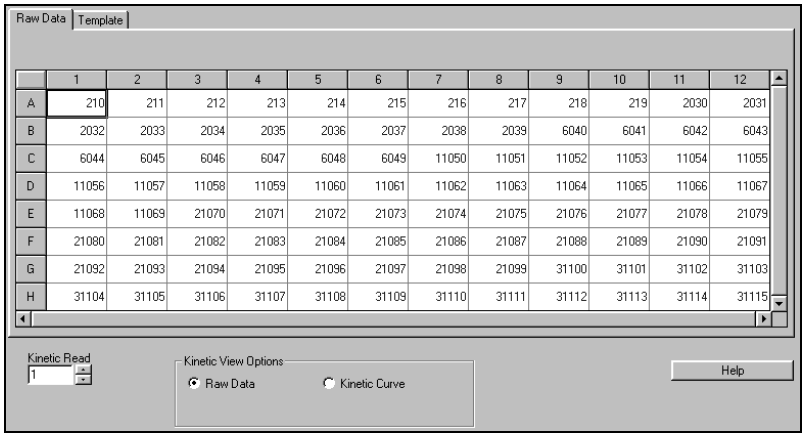
Read Method parameters

Raw Dispense / Read Data

Raw data results and kinetic curves are viewed under the Raw Data tab. To switch between the different views, click the appropriate Kinetic View Options radio button.

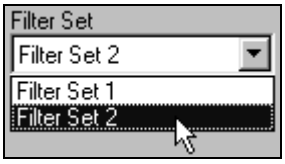
Individual Reads

Raw Data results for individual kinetic reads are shown below:



	1	2	3	4	5	6	7	8	9	10	11	12
A	210	211	212	213	214	215	216	217	218	219	2030	2031
B	2032	2033	2034	2035	2036	2037	2038	2039	6040	6041	6042	6043
C	6044	6045	6046	6047	6048	6049	11050	11051	11052	11053	11054	11055
D	11056	11057	11058	11059	11060	11061	11062	11063	11064	11065	11066	11067
E	11068	11069	21070	21071	21072	21073	21074	21075	21076	21077	21078	21079
F	21080	21081	21082	21083	21084	21085	21086	21087	21088	21089	21090	21091
G	21092	21093	21094	21095	21096	21097	21098	21099	31100	31101	31102	31103
H	31104	31105	31106	31107	31108	31109	31110	31111	31112	31113	31114	31115

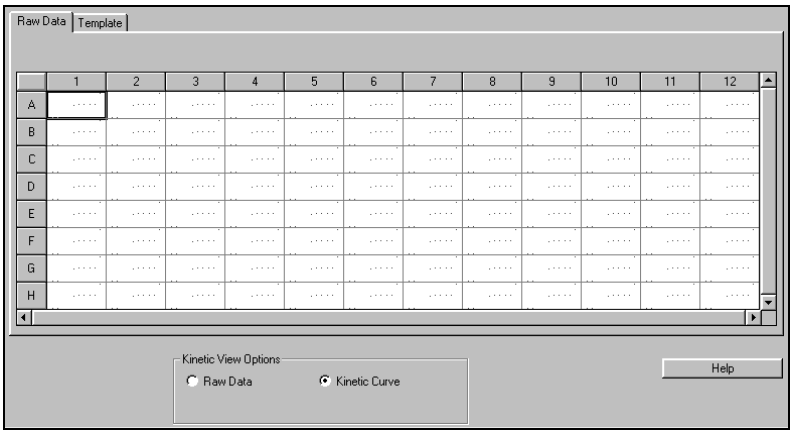
Data is initially reported in this format, reporting the raw values for each well of Kinetic Read #1.



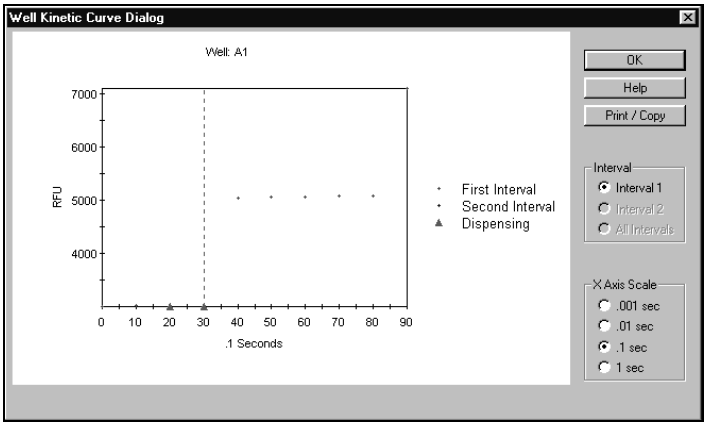
- Each well contains the RFU or RLU value calculated for the the *first* filter set defined in the protocol. If *two* filter sets are defined, use the Filter Set drop-down menu to choose a different filter set.
- The word **'Dispense'** in a well indicates that a dispense or dispense-related activity occurred (well mode only).
- To view the results for a different kinetic read, enter the desired read number in the **Kinetic Read** field (or click the up/down arrow buttons).

Kinetic Curves (Dispense/Read)

Kinetic read results are shown below, in the Kinetic Curve format:



- Each well initially displays a graph of the data points corresponding to the RFU/RLU readings taken over time.
- To **enlarge** the view of the curve in any well, click once on the desired well.



  - Place the cursor over a **data point** to see its RFU/RLU value and reading Time.
  - Depending on the current Dispense/Read parameters, you can adjust the **X Axis Scale** and/or select an **Interval** (sampling window) to view.
  - A **dotted green line** indicates the start of a dispense.
  - A **green triangle** indicates that a dispense or dispense-related activity occurred (well mode only).

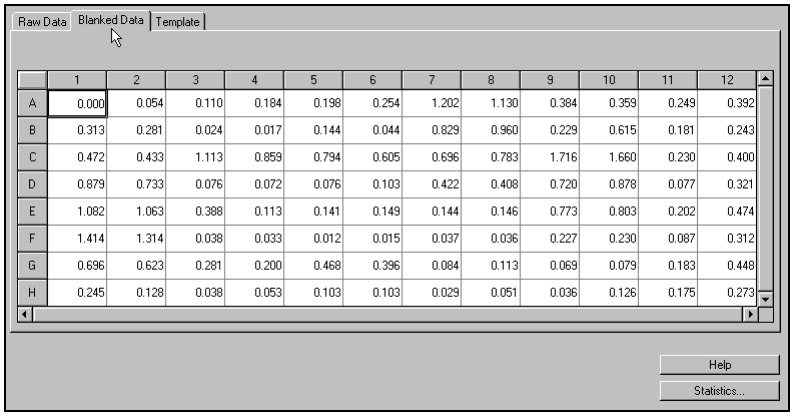
Blanked Data

Blanked Data represents Raw Data that has undergone blank subtraction. The format of the Blanked Data results display differs based on the defined **Read Method** – Endpoint, Kinetic, MultiWavelength, or Spectral Scan.

Note: To view Blanked data for a **Kinetic** or **Spectral Scan** read, click the Raw Data tab, then click the Blanked Data radio button under Kinetic or Scan View Options. See "Raw and Blanked Kinetic Read Data" on page 7-5 or "Raw and Blanked Spectral Data" on page 7-9 for more information.

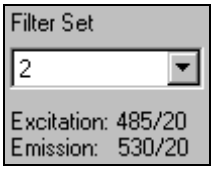
Blanked Endpoint Read Data

Blanked Data results for an **Endpoint** read are shown below:



	1	2	3	4	5	6	7	8	9	10	11	12
A	0.000	0.054	0.110	0.184	0.198	0.254	1.202	1.130	0.384	0.359	0.249	0.392
B	0.313	0.281	0.024	0.017	0.144	0.044	0.829	0.960	0.229	0.615	0.181	0.243
C	0.472	0.433	1.113	0.859	0.794	0.605	0.696	0.783	1.716	1.660	0.230	0.400
D	0.879	0.733	0.076	0.072	0.076	0.103	0.422	0.408	0.720	0.878	0.077	0.321
E	1.082	1.063	0.388	0.113	0.141	0.149	0.144	0.146	0.773	0.803	0.202	0.474
F	1.414	1.314	0.038	0.033	0.012	0.015	0.037	0.036	0.227	0.230	0.087	0.312
G	0.696	0.623	0.281	0.200	0.468	0.396	0.084	0.113	0.069	0.079	0.183	0.448
H	0.245	0.128	0.038	0.053	0.103	0.103	0.029	0.051	0.036	0.126	0.175	0.273

- If a **single-wavelength absorbance** read was performed, each well contains the OD measured at the **Primary** wavelength, less the average of the blank wells.
- If a **dual-wavelength absorbance** read was performed, each well contains the *delta OD* value calculated by the reader, less the average of the blank wells.

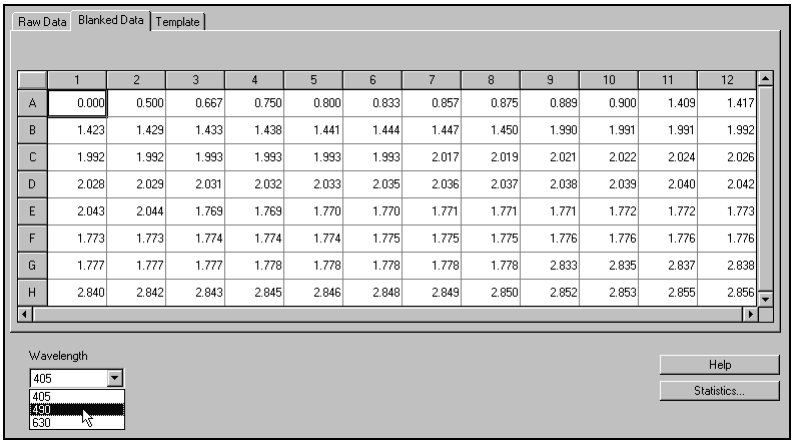


- For **fluorescence** and **luminescence** reads, 1 to 6 sets of data are presented, depending on the number of filter sets specified in the protocol.

  Initially, the RFU/RLU values for filter set #1 are presented. Use the drop-down menu at the bottom of the screen to choose a different filter set.

Blanked MultiWavelength Data

Blanked Data results for a **MultiWavelength** read are shown below:



	1	2	3	4	5	6	7	8	9	10	11	12
A	0.000	0.500	0.667	0.750	0.800	0.833	0.857	0.875	0.889	0.900	1.409	1.417
B	1.423	1.429	1.433	1.438	1.441	1.444	1.447	1.450	1.990	1.991	1.991	1.992
C	1.992	1.992	1.993	1.993	1.993	1.993	2.017	2.019	2.021	2.022	2.024	2.026
D	2.028	2.029	2.031	2.032	2.033	2.035	2.036	2.037	2.038	2.039	2.040	2.042
E	2.043	2.044	1.769	1.769	1.770	1.770	1.771	1.771	1.771	1.772	1.772	1.773
F	1.773	1.773	1.774	1.774	1.774	1.775	1.775	1.775	1.776	1.776	1.776	1.776
G	1.777	1.777	1.777	1.778	1.778	1.778	1.778	1.778	2.833	2.835	2.837	2.838
H	2.840	2.842	2.843	2.845	2.846	2.848	2.849	2.850	2.852	2.853	2.855	2.856

- Each well contains the OD value after **blank subtraction.**
- Use the drop-down list to view Blanked Data for a different wavelength.
- If an **empty plate** is read for a particular wavelength, the empty plate measurements are subtracted from the raw data measurements to generate the **Result** values (e.g. “405 Result”). Blank subtraction is then performed on the Result values.

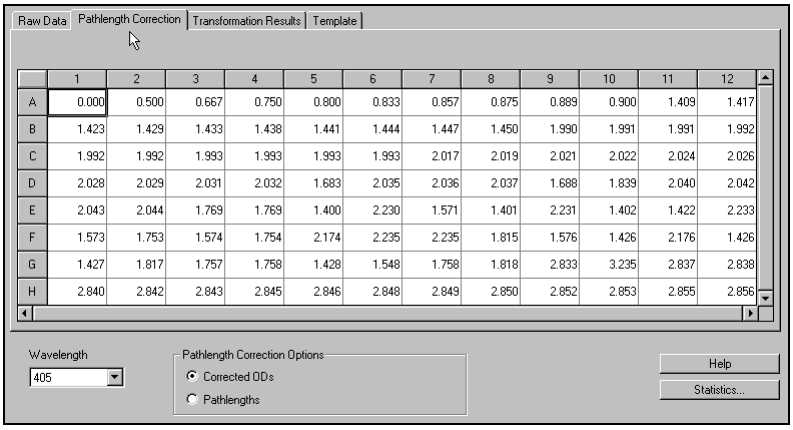
Pathlength Correction

Pathlength Correction can be enabled to normalize absorbances to 1 cm. Its use is controlled within a MultiWavelength protocol’s Read Method dialog. See page 5-16 for more information.

If Pathlength Correction is enabled, KCjunior first calculates the **pathlength** for each well. The absorbance in each well is then divided by the pathlength, resulting in the **corrected absorbance.**

When calculations are complete, the corrected absorbances are displayed under the **Pathlength Correction** results tab.

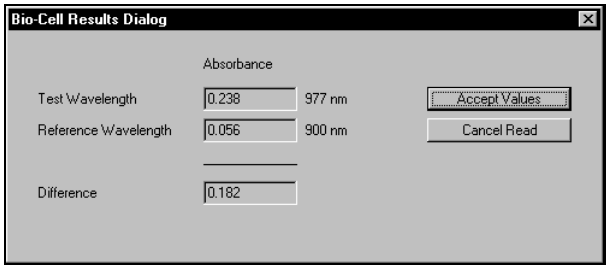
Typical results are shown on the next page:



- The **Corrected ODs** are initially displayed.
- Click the **Pathlengths** radio button to view the pathlength value for each well.
- Each **corrected** well contains the result of the following formula:

  (A[wavelength]well) ÷ pathlength of well

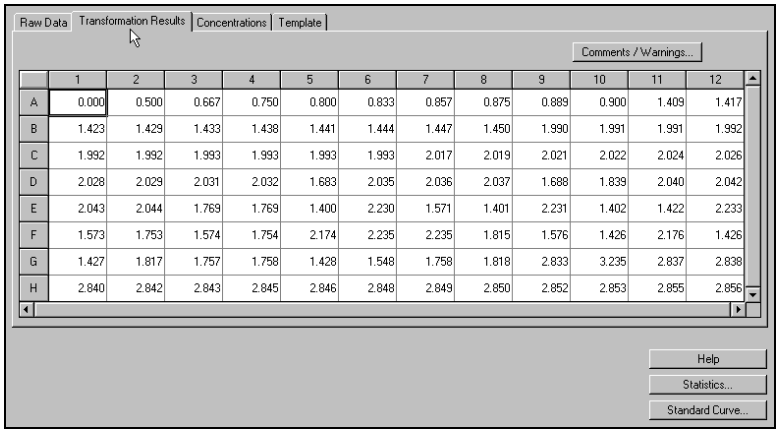
  The method used to calculate pathlengths depends on the Pathlength Correction parameters defined under the **Read Method** tab in the protocol. See page 5-16 for more information.
- To view the corrected absorbances at a different wavelength, choose a value from the **Wavelength** drop-down menu.
- If Bio-Tek's Bio-Cell™ plate was used to calculate the absorbance of water at 1 cm value, click the **Bio-Cell Results** button to see the absorbance readings at the **Test** and **Reference Wavelengths.**



Transformation Results

Transformation Results are displayed if at least one Transformation Formula was applied to one or more wells on the plate.

*Transformation Formulas are applied according to a certain structure. See "Essential Information" in **Getting Started** for more information.*



	1	2	3	4	5	6	7	8	9	10	11	12
A	0.000	0.500	0.667	0.750	0.800	0.833	0.857	0.875	0.889	0.900	1.409	1.417
B	1.423	1.429	1.433	1.438	1.441	1.444	1.447	1.450	1.990	1.991	1.991	1.992
C	1.992	1.992	1.993	1.993	1.993	1.993	2.017	2.019	2.021	2.022	2.024	2.026
D	2.028	2.029	2.031	2.032	1.683	2.035	2.036	2.037	1.688	1.839	2.040	2.042
E	2.043	2.044	1.769	1.769	1.400	2.230	1.571	1.401	2.231	1.402	1.422	2.233
F	1.573	1.753	1.574	1.754	2.174	2.235	2.235	1.815	1.576	1.426	2.176	1.426
G	1.427	1.817	1.757	1.758	1.428	1.548	1.758	1.818	2.833	3.235	2.837	2.838
H	2.840	2.842	2.843	2.845	2.846	2.848	2.849	2.850	2.852	2.853	2.855	2.856

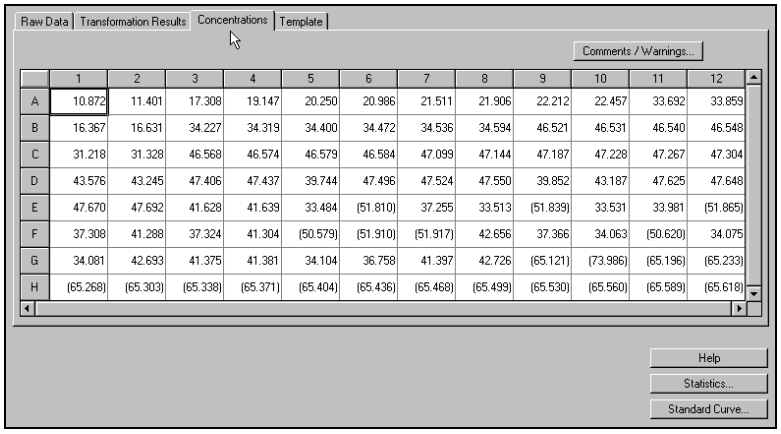
- Each well contains the result of its **Transformation Formula,** if one was applied. Otherwise, the well contains its Raw, Blanked, or Corrected value.
- For **Kinetic** reads, Transformation Formulas are applied to the Maximum Slope.
- For **MultiWavelength** reads, Transformation Formulas are applied to the Raw or Blanked Data for the *first* defined wavelength, unless the **WV** function is used to specify a different wavelength.
- For **multiple Filter Set** reads, Transformation Formulas are applied to the Raw or Blanked Data for the *first* defined filter set, unless the **FS** function is used to specify a different filter set.

  See "Defining Transformations - Formula Syntax" in *Defining Protocols,* for more information on the **WV** and **FS** functions.

Concentrations

Concentrations are reported if curve parameters are defined in the protocol and if the curve generated successfully.

*Concentrations are calculated for every well on the plate, based on the standard curve and according to a certain structure. See "Essential Information" in **Getting Started** for more information.*



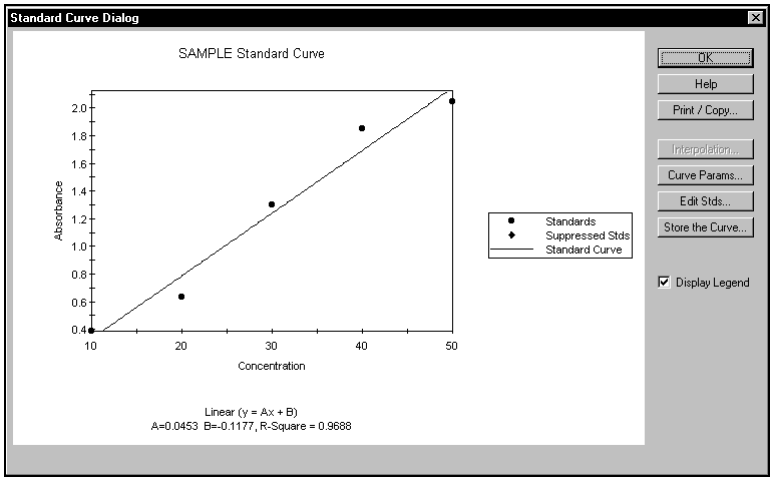
	1	2	3	4	5	6	7	8	9	10	11	12
A	10.872	11.401	17.308	19.147	20.250	20.986	21.511	21.906	22.212	22.457	33.692	33.859
B	16.367	16.631	34.227	34.319	34.400	34.472	34.536	34.594	46.521	46.531	46.540	46.548
C	31.218	31.328	46.568	46.574	46.579	46.584	47.099	47.144	47.187	47.228	47.267	47.304
D	43.576	43.245	47.406	47.437	39.744	47.496	47.524	47.550	39.852	43.187	47.625	47.648
E	47.670	47.692	41.628	41.639	33.484	(51.810)	37.255	33.513	(51.839)	33.531	33.981	(51.865)
F	37.308	41.288	37.324	41.304	(50.579)	(51.910)	(51.917)	42.656	37.366	34.063	(50.620)	34.075
G	34.081	42.693	41.375	41.381	34.104	36.758	41.397	42.726	(65.121)	(73.986)	(65.196)	(65.233)
H	(65.268)	(65.303)	(65.338)	(65.371)	(65.404)	(65.436)	(65.468)	(65.499)	(65.530)	(65.560)	(65.589)	(65.618)

- Each well contains its calculated concentration.
- For **Endpoint** reads, curves are based on the OD, RFU, or RLU values.
- For **Kinetic** reads, curves are based on the Maximum Slope values.
- For **MultiWavelength** reads, curves are based on the measurements at the *first* defined wavelength unless Transformation Formulas utilizing the **WV** function are applied to specify a different wavelength.
- For **multiple Filter Set** reads, curves are based on the measurements at the *first* defined filter set, unless Transformation Formulas utilizing the **FS** function are applied to specify a different filter set.
- See "Defining Transformations - Formula Syntax" in *Defining Protocols*, for more information on the **WV** and **FS** functions.
- Click the **Standard Curve** button to view the graph (see next page).

The Standard Curve

The Standard Curve dialog displays the calculated curve and provides options for printing, copying, and storing the curve, and for editing standard outliers and/or curve parameters:

*To access **advanced graphing options,** place the cursor inside the graph and click the right mouse button.*

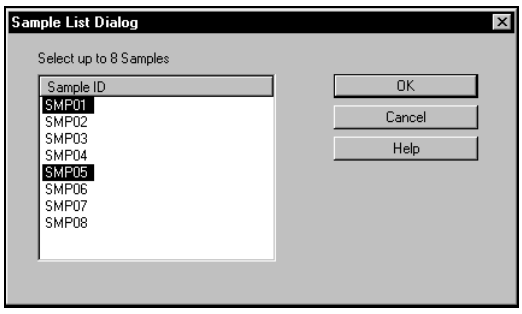


- Click **Print/Copy** to print the graph, or copy it to the Clipboard.
  - A **Curve Report** can be printed if it is selected as a reporting option within the protocol. See page 5-61 for more information.
- If the Calculate Interpolation Value curve parameter is selected in the protocol, click **Interpolation** to see the concentration calculated for the value.
- Click **Curve Params** to change any curve fitting parameters.
- Click **Edit Stds…** to suppress or restore one or more standards. **Note:** This feature is not available for titer or stored curves.

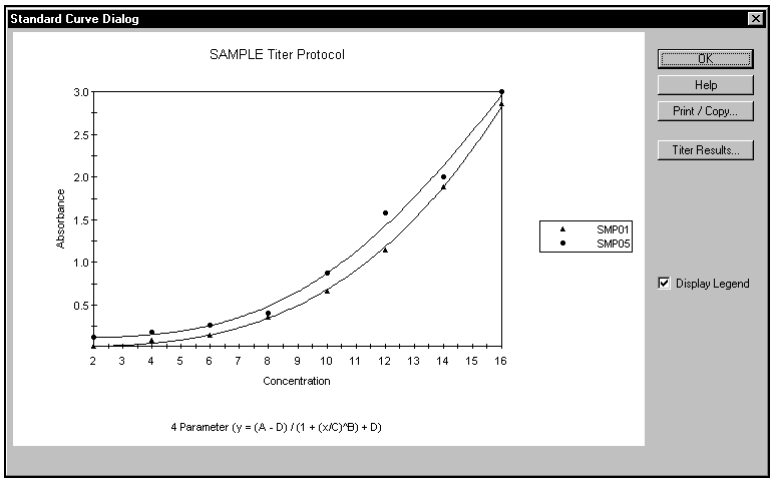
  Alternatively, click on a standard point in the graph to temporarily exclude it from curve calculations. Click again to restore the standard.
- Click **Store the Curve** to store a standard curve for use with other protocols.
- Toggle the **Display Legend** setting to include/exclude a legend in the graph.
- Click **OK** to close the Standard Curve dialog. Any changes to the standards will be reflected in the results calculations.
- Click the **right mouse button** inside the graph to access advanced graphing options.

Titer Curves

If **titer** curves were generated, clicking the Standard Curve button opens the **Sample List** dialog:



- Select up to **8** samples to be overlaid in one graph.
- Press the **Shift** key while using the mouse to select contiguous IDs (ex. SMP01-04).
- Press the **Ctrl** key while using the mouse to select non-contiguous IDs (ex. SMP02, 05, 07, 08).
- Click the **OK** button to view the graph:



➢ If **Graph Interpolation Result** is enabled under **Setup|Configuration|Reporting Options,** the interpolation value will be plotted on the curve.

The value will **not** be reported, however, if more than one sample is selected.

➢ Data points cannot be edited within this dialog. To **exclude** a well from the curve, return to the Raw Data results display and **Mask** the desired well.

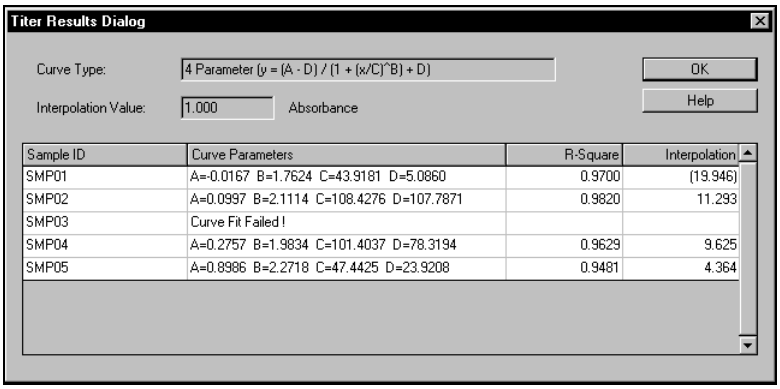
- Click **Print/Copy** to print the graph, or copy it to the Clipboard.

  Note: A Titer curve report can also be printed if it is selected as a reporting option within the protocol.

- Click **Titer Results** to view Curve Parameters, R-Square values, and Interpolation results for all samples on the plate.
- Toggle the **Display Legend** setting to include/exclude a legend in the graph.
- Click the **right mouse button** inside the graph to access advanced graphing options.
- Click **OK** to close the Titer Curve dialog.

Titer Results

Regardless of the number of titer curves selected for viewing, the **Titer Results** dialog shows results for all samples defined for the plate.



- The curve fitting method chosen in the protocol and its equation are displayed under **Curve Type.**
- If defined in the protocol, the **Interpolation Value** is provided.
- Each **Sample ID** is listed in alphabetical order.
- For each sample, the **Curve Parameters** and corresponding **R-Square** values are reported.
- If an interpolation value was defined, its calculated value for each titer curve is reported in the **Interpolation** column.

Interacting with the Graph

The displayed graph can be scaled, moved, and zoomed, and the altered display can then be printed or copied to the Clipboard. The graph report name and and curve equation information does not scale, move, or zoom with the graph.

Note: At any time, press "**r**" to reset the graph to its original size and position.

To **scale** the graph:

1. Press and hold the **Ctrl** key.
2. Press and hold **both** mouse buttons (or the middle button of a 3-button mouse).
3. Move the mouse down to increase the graph size, or up to decrease the graph size.

To **move** the graph:

1. Press and hold the **Shift** key.
2. Press and hold **both** mouse buttons (or the middle button of a 3-button mouse).
3. Move the mouse to change the position of the graph.

To **zoom** in on a portion of the graph:

1. Press and hold the **Ctrl** key.
2. Press and hold the **left** mouse button.
3. Drag the mouse to select the zoom area.
4. Release the mouse button before releasing the Ctrl button.

To **zoom** in on a portion of the graph and have the axes adjust accordingly:

1. Press and hold the **Shift** key.
2. Press and hold the **left** mouse button.
3. Drag the mouse to select the zoom area.
4. Release the mouse button before releasing the Shift button.

Advanced Graphing Options

Clicking the **right mouse button** from within the graph provides access to many advanced graphing options, including:

- Creating a **border** around the entire chart.
- Changing the **color** of the background, foreground, lines, and symbols.
- **Scaling** the X and/or Y axis to better focus in on the curve.

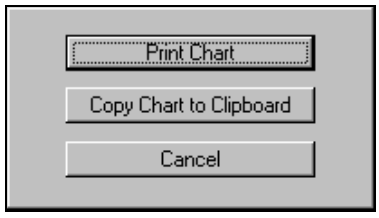
- Changing the **title** and **font** of the X and/or Y axis.
- Adding a **legend** to the graph.
- Adding informative **labels** to the graph.

Printing and Copying the Graph

The graph and any edits made to it can be copied to the clipboard or printed.

To **print** or **copy** the graph:

1. Click the **Print/Copy** button. A selection list will appear:



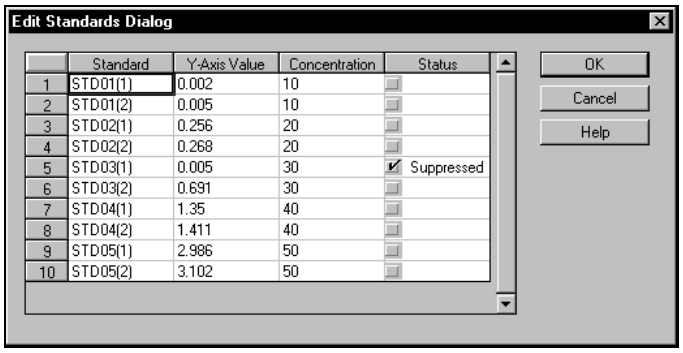
2. Click the **Print Chart** button to print the graph.
3. Click the **Copy Chart to Clipboard** button to copy the graph to the Clipboard. The graph can then be pasted into another program.
4. Click **Cancel** to return to the graph.

Editing the Standard Curve

After the standard curve has been generated, one or more standards can be temporarily excluded from the recalculation of the curve. This feature does **not** apply to titer curves or stored curves.

There are two ways to **edit** the standard curve:

1. From within the Standard Curve dialog, click once on the standard point to be excluded. The selected standard will dim, and the curve will automatically attempt to recalculate.
   - **Restore** an edited standard by clicking on it once. The curve will automatically recalculate.
2. Click the **Edit Stds…** button to open the Edit Standards Dialog. This dialog lists each standard with its Y-Axis Value, Concentration (as defined in the protocol), and current Status.



- If the **Status** field is empty, the standard is currently included in the curve calculation. Click the checkbox to suppress the standard.
- If the **Status** field contains "Suppressed" the standard is currently suppressed. Click the checkbox to restore the standard.
- Click **OK** to close the dialog and return to the Standard Curve dialog. The curve will automatically recalculate.

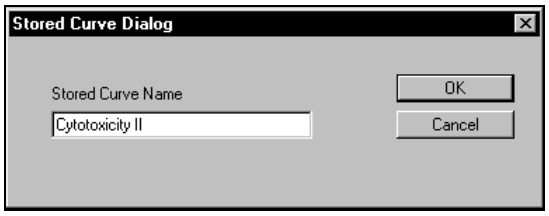
Storing the Standard Curve

The standard curve can be stored for use by another protocol. This feature does **not** apply to titer curves.

To **store** a standard curve:

1. From within the Standard Curve dialog, click the **Store the Curve** button.

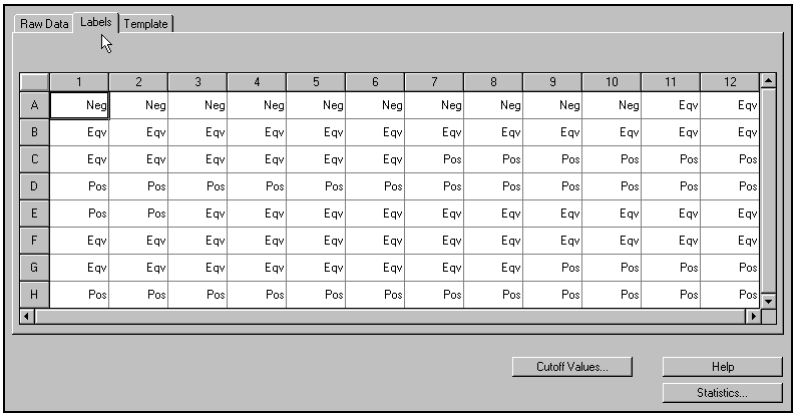
   The **Stored Curve** dialog will appear.



2. Enter a unique name and click **OK** to store the curve and return to the Standard Curve dialog.
3. Click **OK** to close the Standard Curve dialog and return to the Concentrations results display.

Labels

Labels are displayed if cutoffs are defined in the protocol.



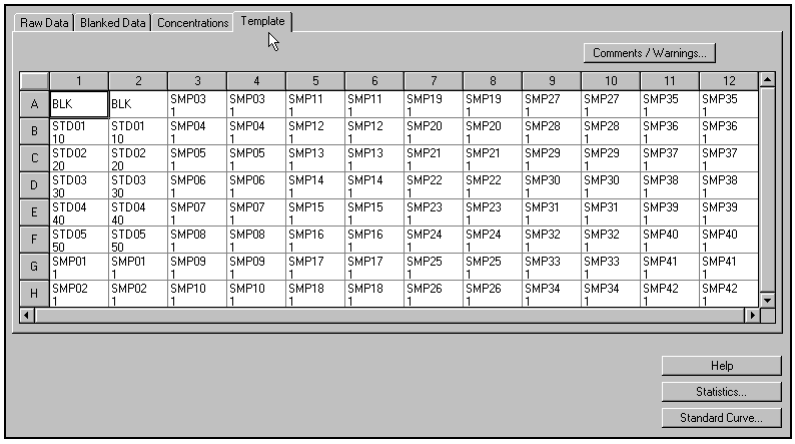
	1	2	3	4	5	6	7	8	9	10	11	12
A	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Eqv	Eqv
B	Eqv	Eqv	Eqv	Eqv	Eqv	Eqv	Eqv	Eqv	Eqv	Eqv	Eqv	Eqv
C	Eqv	Eqv	Eqv	Eqv	Eqv	Eqv	Pos	Pos	Pos	Pos	Pos	Pos
D	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos
E	Pos	Pos	Eqv	Eqv	Eqv	Eqv	Eqv	Eqv	Eqv	Eqv	Eqv	Eqv
F	Eqv	Eqv	Eqv	Eqv	Eqv	Eqv	Eqv	Eqv	Eqv	Eqv	Eqv	Eqv
G	Eqv	Eqv	Eqv	Eqv	Eqv	Eqv	Eqv	Eqv	Pos	Pos	Pos	Pos
H	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos

- Each well contains the **Label** assigned to it after cutoff evaluation.
- For **Endpoint** reads, cutoff evalution is based on the OD, RFU, or RLU values.
- For **Kinetic** reads, evaluation is based on the Maximum Slope values.
- For **MultiWavelength** reads, evaluation is based on the measurements at the *first* defined wavelength unless Transformation Formulas utilizing the **WV** function are applied to specify a different wavelength.
- For **multiple Filter Set** reads, evaluation is based on the measurements at the *first* defined filter set, unless Transformation Formulas utilizing the **FS** function are applied to specify a different filter set.

  See “Defining Transformations - Formula Syntax” in *Defining Protocols,* for more information on the **WV** and **FS** functions.
- Click the **Cutoff Values** button to see the cutoff criteria, the calculated cutoff value(s), and the labels assigned to each cutoff zone.

Template

Well identification information can be viewed under the **Template** tab.

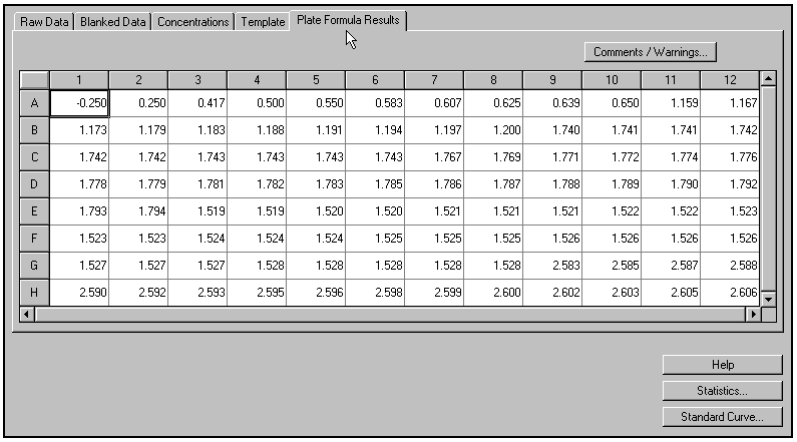


	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	BLK	SMP03 1	SMP03 1	SMP11 1	SMP11 1	SMP19 1	SMP19 1	SMP27 1	SMP27 1	SMP35 1	SMP35 1
B	STD01 10	STD01 10	SMP04 1	SMP04 1	SMP12 1	SMP12 1	SMP20 1	SMP20 1	SMP28 1	SMP28 1	SMP36 1	SMP36 1
C	STD02 20	STD02 20	SMP05 1	SMP05 1	SMP13 1	SMP13 1	SMP21 1	SMP21 1	SMP29 1	SMP29 1	SMP37 1	SMP37 1
D	STD03 30	STD03 30	SMP06 1	SMP06 1	SMP14 1	SMP14 1	SMP22 1	SMP22 1	SMP30 1	SMP30 1	SMP38 1	SMP38 1
E	STD04 40	STD04 40	SMP07 1	SMP07 1	SMP15 1	SMP15 1	SMP23 1	SMP23 1	SMP31 1	SMP31 1	SMP39 1	SMP39 1
F	STD05 50	STD05 50	SMP08 1	SMP08 1	SMP16 1	SMP16 1	SMP24 1	SMP24 1	SMP32 1	SMP32 1	SMP40 1	SMP40 1
G	SMP01 1	SMP01 1	SMP09 1	SMP09 1	SMP17 1	SMP17 1	SMP25 1	SMP25 1	SMP33 1	SMP33 1	SMP41 1	SMP41 1
H	SMP02 1	SMP02 1	SMP10 1	SMP10 1	SMP18 1	SMP18 1	SMP26 1	SMP26 1	SMP34 1	SMP34 1	SMP42 1	SMP42 1

- The **Well ID** or **Sample ID** and corresponding **concentration/dilution** value are displayed for each well.
- **Well IDs** are used to identify the four well types supported by KCjunior – Blank, Standard, Sample, and Control.

Plate Formula Results

Plate Formula Results are displayed if a Plate Formula is defined in the protocol.



	1	2	3	4	5	6	7	8	9	10	11	12
A	-0.250	0.250	0.417	0.500	0.550	0.583	0.607	0.625	0.639	0.650	1.159	1.167
B	1.173	1.179	1.183	1.188	1.191	1.194	1.197	1.200	1.740	1.741	1.741	1.742
C	1.742	1.742	1.743	1.743	1.743	1.743	1.767	1.769	1.771	1.772	1.774	1.776
D	1.778	1.779	1.781	1.782	1.783	1.785	1.786	1.787	1.788	1.789	1.790	1.792
E	1.793	1.794	1.519	1.519	1.520	1.520	1.521	1.521	1.521	1.522	1.522	1.523
F	1.523	1.523	1.524	1.524	1.524	1.525	1.525	1.525	1.526	1.526	1.526	1.526
G	1.527	1.527	1.527	1.528	1.528	1.528	1.528	1.528	2.583	2.585	2.587	2.588
H	2.590	2.592	2.593	2.595	2.596	2.598	2.599	2.600	2.602	2.603	2.605	2.606

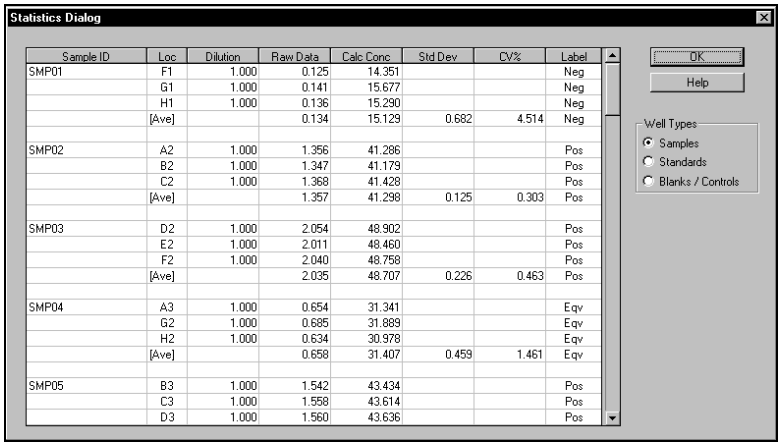
- Each well contains the result of the **Plate Formula** defined in the protocol.
- For **Endpoint** reads, the Plate Formula is based on the OD, RFU, or RLU values.
- For **Kinetic** reads, the Plate Formula is based on the Maximum Slope values.
- For **MultiWavelength** reads, Plate Formulas are applied to the Raw or Blanked Data for the *first* defined wavelength, unless the **WV** function is used to specify a different wavelength.
- For **multiple Filter Set** reads, Plate Formulas are applied to the Raw or Blanked Data for the *first* defined filter set, unless the **FS** function is used to specify a different filter set.

  See “Defining Transformations - Formula Syntax” in *Defining Protocols*, for more information on the **WV** and **FS** functions.

Statistics

Within each results display, the **Statistics** button is available if a Well ID Template is defined in the protocol.

When the **Statistics** button is clicked, KCjunior automatically calculates and displays the mean, standard deviation, and coefficient of variation of every replicate group.



Sample ID	Loc	Dilution	Raw Data	Calc Conc	Std Dev	CV%	Label
SMP01	F1	1.000	0.125	14.351			Neg
	G1	1.000	0.141	15.677			Neg
	H1	1.000	0.136	15.290			Neg
	[Ave]		0.134	15.129	0.682	4.514	Neg
SMP02	A2	1.000	1.356	41.286			Pos
	B2	1.000	1.347	41.179			Pos
	C2	1.000	1.368	41.428			Pos
	[Ave]		1.357	41.298	0.125	0.303	Pos
SMP03	D2	1.000	2.054	48.902			Pos
	E2	1.000	2.011	48.460			Pos
	F2	1.000	2.040	48.758			Pos
	[Ave]		2.035	48.707	0.226	0.463	Pos
SMP04	A3	1.000	0.654	31.341			Eqv
	G2	1.000	0.685	31.889			Eqv
	H2	1.000	0.634	30.978			Eqv
	[Ave]		0.658	31.407	0.459	1.461	Eqv
SMP05	B3	1.000	1.542	43.434			Pos
	C3	1.000	1.558	43.614			Pos
	D3	1.000	1.560	43.636			Pos

- Click the **Samples, Statistics,** or **Controls** button to see a different set of statistics, or click **OK** to return to the results display.
- For **MultiWavelength** reads, the Raw Data column contains the Read (or Result, if an empty plate was read) values for the *first* defined wavelength. The Blank Data column contains the blanked data for the *first* defined wavelength.
- For **multiple Filter Set** reads, the Raw and Blank Data columns contain the raw and blanked values for the *first* defined filter set.
- If viewing statistics for a titer protocol, click the **Titer Results** button to view curve parameters, r-square values, and interpolation results for all samples on the plate.
- The information provided in the **Statistics** dialog depends on the protocol parameters. The following may be displayed:

  Well ID — The **identifier** assigned to each well.

  Loc — The **location** of the Well ID in the plate.

  Note: If viewing statistics for a protocol that uses a stored curve, the **Loc** values for the standards will contain XXX.

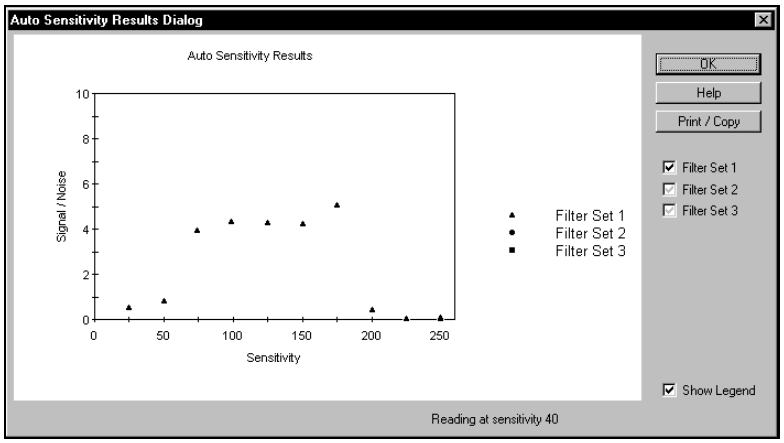
Blank Data	If the protocol specifies blanking and does not contain transformation formulas, the **Blank Data** will be displayed.
Calc Conc	If concentrations were calculated, they will be displayed in the **Calc Conc** column.
Corrected	If Pathlength Correction is enabled in the protocol (MultiWavelength reads only) to normalize absorbances to 1 cm, the **Corrected** ODs will be displayed.
Concentration	If viewing statistics for a protocol that uses a stored curve, the concentration values (as defined in the protocol) will be displayed in the **Concentration** column.
Dilution	If dilutions were assigned in the protocol, they will be displayed in the **Dilutions** column.
Label	If cutoff criteria are specified, the **Label** will be displayed for every replicate and group mean.
Max Slope	The **Max Slope** is reported for kinetic protocols.
Mean	The **Mean** is automatically calculated for every multiple-replicate standard, control, blank, and sample group. **Note:** The **Mean** is not displayed for samples/controls with multiple dilutions.
Raw Data	If the protocol does not specify blanking or contain transformation formulas, the **Raw Data** will be displayed.
Std Dev, CV%	The **Standard Deviation** and **CV%** are automatically calculated for every multiple-replicate standard, control, blank, and sample group. **Note:** CV% = 100*(Std Dev/Mean)
Transform	If the protocol contains transformation formulas, the results will be displayed in the **Transform** column.
Y-Axis Data	If viewing statistics for a protocol that uses a stored curve, the **Y-Axis Data** will be displayed for the standards.

Auto Sensitivity Results

For fluorescence and luminescence readers, KCjunior provides an **Automatic Sensitivity Adjustment** feature as an alternative to the more manual method of defining multiple filter sets with varying sensitivity settings. See "Defining Filter Sets" under *Defining Protocols* for more information. If this feature is enabled, KCjunior instructs the reader to perform several pre-readings on the microplate. In general, these readings are performed on user-specified wells using incremental sensitivity settings. Each increase in sensitivity raises the RFU/RLU values of all of the wells.

If the Automatic Sensitivity Adjustment option **Scale to High/Low Range** is selected, KCjunior compares the results of the pre-readings and selects the sensitivity that gives the best signal-to-noise ratio when comparing one (higher) set of wells with another (lower) set of wells. The entire microplate is then read using this sensitivity setting.

A graph showing the results of these pre-readings can be viewed from the results dialog, by clicking the **Auto Sensitivity** button. The Auto Sensitivity Results dialog appears:



- This graph can be viewed during or following the automatic sensitivity adjustment process.
- Each point represents the **signal-to-noise ratio** calculated at a particular sensitivity setting.
- To change the filter set(s) shown in the graph, select or deselect the **Filter Set** checkboxes.
- This graph is **not** saved with the plate. Click **Print/Copy** to print the graph or to copy/paste it into another application.

- If the message “There is no valid auto sensitivity data to display” appears, an error occurred during the auto sensitivity adjustment process. See “Comments and Warnings” in the *Troubleshooting* section of this guide for more information.

Automatic Sensitivity Adjustment Process

The automatic sensitivity adjustment process (for the Scale to Low/High Range option) is performed as follows:

- Using the filter set parameters provided, an initial reading is performed on the designated “pre-reading” wells, using a sensitivity setting of 25.
- A signal-to-noise ratio is calculated. If it is found to be valid, the sensitivity setting and the value are retained for further evaluation.
- The sensitivity is then increased by increments of 25 and additional readings are made until one of the following conditions are met:

  Condition 1 (success)

  If two successive valid signal-to-noise ratios are within either 0.1 or 5 percent of each other, then 2 additional readings are performed using sensitivity settings at one-quarter increments between the sensitivity of those readings. For example, if the signal-to-noise ratio at sensitivities 50 and 75 are 120.31 and 120.38, respectively, then additional readings will be performed at sensitivities 55, 60, 65, and 70.

  The valid signal-to-noise ratios of the 6 successive readings are then compared to determine the highest value. The sensitivity of the highest signal-to-noise ratio is then chosen for the actual filter set read. If the highest signal-to-noise ratio occurs at multiple sensitivity settings, then the highest sensitivity will be selected.

  Condition 2 (success)

  If Condition 1 is not met after a sensitivity reading of 250 or before the sensitivity at which one or more of the High Wells exceeds the High Value, then the sensitivity of the highest valid signal-to-noise ratio acheived is chosen for the actual filter set read. If the highest signal-to-noise ratio occurs at multiple sensitivity readings, then the highest sensitivity is selected.

  Condition 3 (failure)

  If Condition 2 is not met because there are no valid signal-to-noise ratios, then an error message will appear and the read will be terminated.

Condition 4 (failure)

If none of the previous conditions are met, then an error message will appear and the read will be terminated.

Signal-to-Noise Ratio Calculation

- If there are *multiple* Low Wells and Test Wells defined in the protocol, the signal-to-noise ratio is calculated as follows:

  (Mean of the Test Wells - Mean of the Low Wells) / Total Standard Deviation

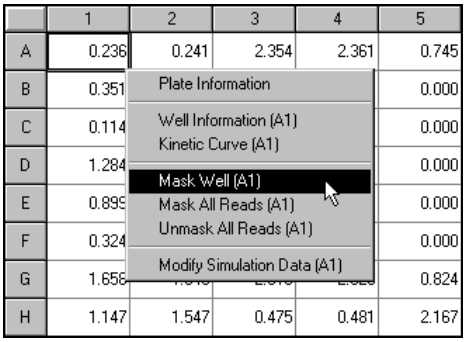
  where Total Standard Deviation = SQRT((Std Dev of the Test Wells)^2 + (Std Dev of the Low Wells)^2))

- If there is a *single* Low Well and/or a *single* Test Well, the signal-to-noise ratio is calculated as follows:

  (Mean of the Test Wells - Mean of the Low Wells) / Mean of the Low Wells

Results Options

From within any results matrix, highlighting a well and clicking the **right mouse button** produces a sub-menu with a variety of options:



Accessibility to some Results Options depends on the protocol definition, the current results display, and the method of obtaining plate data. For example, "Modify Simulation Data" is not available if the plate data was retrieved from a microplate reader.

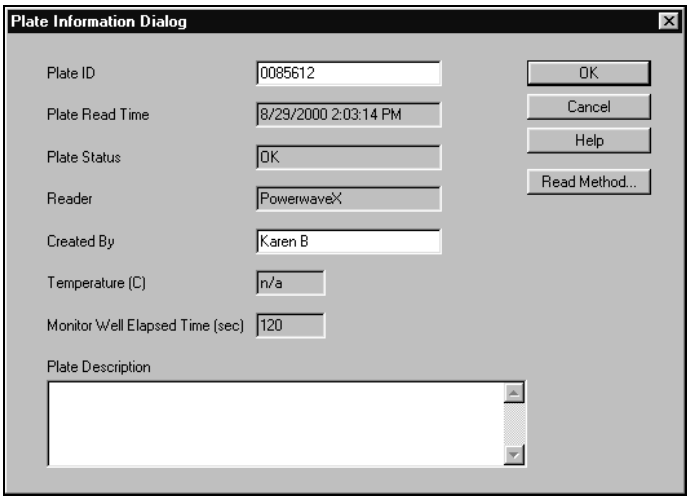
All **Results Options** are described below and on the following pages.

Plate Information

Detailed **Plate Information** is available from within any results display. To access this information:

1. Highlight any well in any results matrix.
2. Click the right mouse button. The **Results Options** menu will appear.

3. Select **Plate Information.** The **Plate Information** dialog appears:



- The **Plate ID** is an optional identifier associated with the plate. It is entered at run time, either manually or via a reader's plate barcode scanner. The ID can be changed here if the result file has not yet been saved.
- The date and time of the plate reading is displayed in the **Plate Read Time** field.
- The possible **Plate Status** values are:

  OK if the plate was read without error.

  Aborted if the plate read was aborted before completion.

  Reader Control Error if the reader experienced a failure before the completion of the read and could no longer respond to KCjunior.

  Reader Error [code] if the reader experienced a failure before the completion of the read and was able to communicate an error code.
- The **Reader** field contains the reader model with which the plate was read.
- The name of the person to whom KCjunior is registered appears as the default in the **Created By** field. The name can be changed here if the plate has not yet been saved.
- If applicable, the **Temperature** will be reported, in degrees Celsius. This value reflects the temperature of the incubation chamber at the time the plate was read.
- If the **Monitor Well** feature was enabled in the protocol, the dialog displays the elapsed time between the start of the

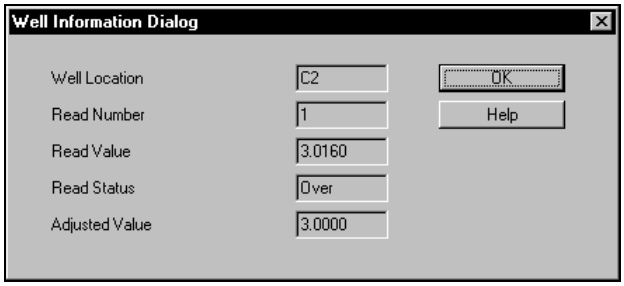
monitoring process and the point when the monitored well(s) met the specified criteria.

- The **Plate Description** can be defined or edited in this dialog.
- To see the Read Method parameters defined for this plate, click the **Read Method** button.
- Click **OK** to save any changes and return to the previous dialog.
- Click **Cancel** to close this dialog without saving changes.

Well Information

Detailed **Well Information** is available for any well, from within the Raw Data results display. To access this information for a particular well:

1. Click the **Raw Data** results tab.
2. Place the cursor over the desired well and click the right mouse button. The **Results Options** menu will appear.
3. Select **Well Information.** The Well Information dialog will appear:



- The **Well Location** represents the well's location in the microplate, in row by column format.
- For a multiple-plate Endpoint read, the **Read Number** represents the plate for which the raw data value in this well was measured.
- If the read is Kinetic, the **Kinetic Read** represents the read during which the raw data value in this well was measured.
- For a MultiWavelength read, the **Wavelength** field shows the wavelength associated with the current Read Value, and Empty, Read, or Result, as appropriate for the data set.
- The **Read Value** is the original unchanged Raw Data value.

  Note: A Read Value of -99.99 or -99,999 indicates a well error (see Read Status, **Error** on the next page).

- The possible **Read Status** values are:

  OK if the well was read without error.

  Out if the Read Value falls outside the acceptable min/max data range and Discard Out of Range Values is selected.

  Over if the Read Value exceeds the upper limit of the acceptable min/max data range and Discard Out of Range Values is not selected.

  Under if the Read Value falls below the lower limit of the acceptable min/max data range and Discard Out of Range Values is not selected.

  Error if the *reader* determined the OD, RFU, or RLU of the well to be significantly out-of-range.

- If the Read Status is Over or Under, the **Adjusted Value** contains the Raw Data Limits or Blank Limits Maximum or Minimum Value as defined in the protocol.
- Click **OK** to close the dialog and return to the results display.

Masking Wells

Within any results display, individual wells can be **masked,** or temporarily excluded, from a recalculation of the results. For example, a standard replicate with an atypical absorbance can be temporarily masked and excluded from the recalculation of the standard curve.

To **Mask** a well:

1. Place the cursor over the desired well and click the right mouse button once. The **Results Options** menu will appear.
2. Select **Mask Well** to mask the current well.

   If the read is **kinetic,** the well will be masked only in the current kinetic read. Select **Mask All Reads** to mask the current well across all kinetic reads.

   - In the **Raw Data** results display, the value in a masked well appears with a line running through it (ex. ~~1.275~~).
   - In other results displays, the well will be **empty.**

To **Unmask** a well:

1. Place the cursor over the desired well and click the right mouse button. The **Results Options** menu will appear.
2. Select √ **Mask Well** to unmask the current well.

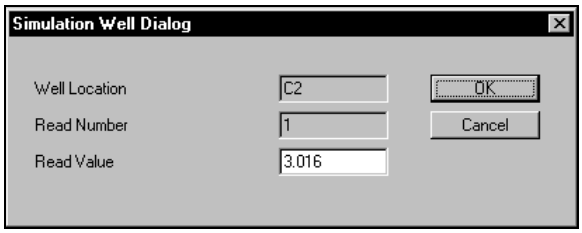
   If the read is **kinetic,** the well will only be unmasked in the current kinetic read. Select **Unmask All Reads** to unmask the current well from all kinetic reads.

Modifying Simulation Data

If results were generated based on manually entered measurements, the measurements can be **modified** from within the Raw Data results display.

To modify manually-entered (simulated) data:

1. Click the **Raw Data** tab.
2. Place the cursor over the desired well and click the right mouse button once. The **Results Options** menu will appear.
3. Select **Modify Simulation Data.** The **Simulation Well** dialog will appear:



   - The **Well Location** represents the well's location in the microplate, in row x column format.
   - If the read is kinetic, the **Read Number** represents the read during which the value for this well was measured.
   - The **Read Value** is the current value in the well.

4. Edit the **Read Value,** then click **OK** to return to the Raw Data results display.

Printing Reports

Reports

After plate data has been acquired and data reduction is complete, KCjunior can generate a user-customizable set of reports. Available reports include the Protocol, Raw Data, Maximum Slope, Formula, Column, and Curve Reports, as well as the configurable Matrix Report.

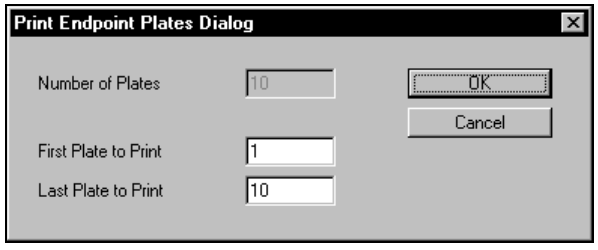
Reporting options are selected and customized within a protocol, under the **Report** tab. See "Defining Protocol Parameters - Reports" in *Defining Protocols* for more information.

Note: A protocol must be open to print most reports. If no protocol is open, however, a Raw Data Report containing just the raw plate data can be printed.

Print Preview

Before actually printing reports, select **Results|Print Preview** to see what they will look like.

- For multiple-plate endpoint reads, the **Print Endpoint Plates** dialog appears.



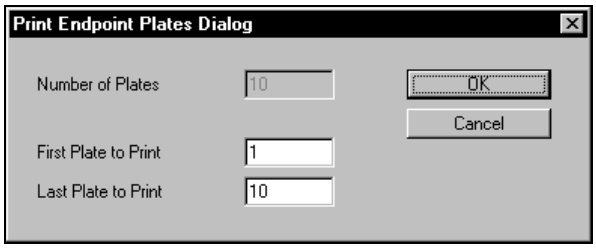
  - The **Number of Plates** field contains the total number of plates that were read.
  - Adjust the **First Plate to Print** and **Last Plate to Print** values to preview a subset of the total number of plates.
  - Click **OK** to continue with the preview.

- The **Print Preview** dialog can display one or two pages at a time.
  - Click Next Page to view the next page of the report.
  - Click Prev Page to view the previous page.
  - Click Zoom In or Zoom Out to change the view.
  - Click Print... to access the **Print** dialog, to select a printer and related options.
  - Click Close to close the print preview dialog and return to the KCjunior main screen.

Print Results

Select **Results|Print Results** to print the reports.

- If printing reports for a multiple-plate endpoint read, the **Print Endpoint Plates** dialog appears.

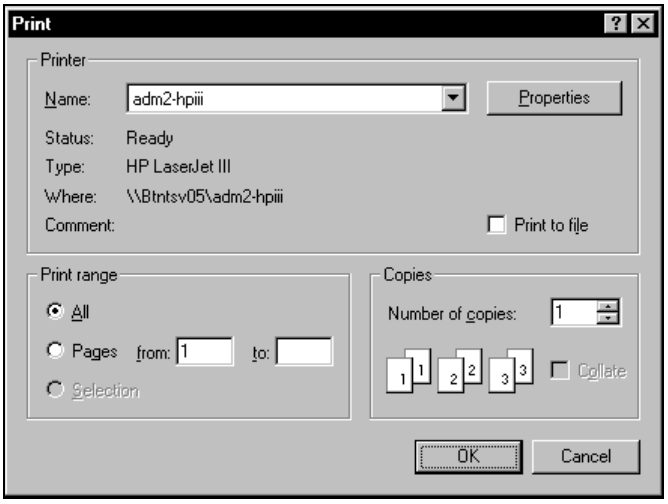


  - The **Number of Plates** field contains the total number of plates that were read.
  - Adjust the **First Plate to Print** and **Last Plate to Print** values to preview a subset of the total number of plates.
  - Click **OK** to continue with the preview.

Print Setup

To change the printer, paper size or source, or page orientation, select **Results|Print Setup** to access a standard Windows Print Setup dialog.

The **Print Setup** dialog appears:



- To select a different printer, click the **Printer Name** drop-down arrow.
- To change print properties such as paper type and resolution, click the **Properties** button.
- To select a different paper size, click the **Paper Size** drop-down arrow.
- To select a different paper source, click the **Paper Source** drop-down arrow.
- To select the paper orientation, click the **Portrait** or **Landscape** radio button.
- Click **OK** to save the changes and return to the KCjunior main screen.

Utilities

Overview

Special reader and database maintenance services are accessible through the **Utilities** menu, including:

- Incubation Control (reader-dependent)
- Carrier Out/In (reader-dependent)
- Dispenser Control Panel
- Reader System Test
- Universal Plate Test
- Database Maintenance functions:
  - Creating empty Results and Protocol Databases for archiving.
  - Archiving results records, protocols, stored curves, and reader system and universal plate tests.
  - Deleting protocols from the current Protocol Database.
  - Deleting results records, stored curves, and reader system and universal plate tests from the current Results Database.

Incubation Control

If the attached reader supports incubation, the **Incubation Control** utility is available.

Use this utility to set the incubation setpoint temperature and duration, then to turn on the reader's incubator and allow it to stabilize before performing a plate read.

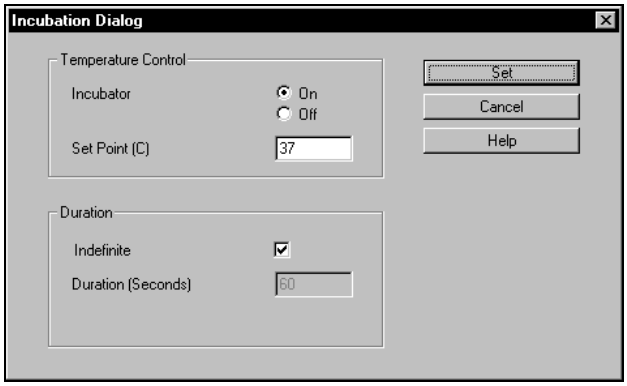
- **Note:** For the EL312I and EL340I readers, the Incubation Control utility does not set or monitor the reader temperature. This utility can, however, be used as a **timer** while the incubation temperature rises to and settles at a setpoint. See step 6 for more information.

An alternative method for controlling incubation is to define incubation information in a **protocol.** If the protocol is open, KCjunior will automatically turn on the reader's incubator when a plate read is initiated. The plate read will begin when the incubation temperature reaches and stabilizes at the setpoint temperature.

To use the **Incubation Control** utility:

1. Ensure that the reader is properly connected to the computer, and that all communication parameters are set correctly.
2. Select **Utilities|Incubation Control** .

   The **Incubation Control** dialog appears:

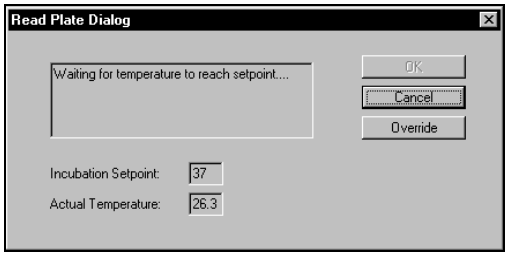


*Incubate a plate **now**.*

3. To turn the incubator **on** and incubate a plate *now*:
   - Click the **On** radio button.
   - Enter **the Set Point** temperature.

     The valid range for most readers is from 20°C (or 4°C above room temperature, whichever is greater), to 50°C.
   - Deselect **Indefinite** duration.
   - Enter the incubation **Duration** in seconds.

- Click **Start.** The **Read Plate** dialog appears, showing the **Actual Temperature** of the reader as it rises toward the setpoint.

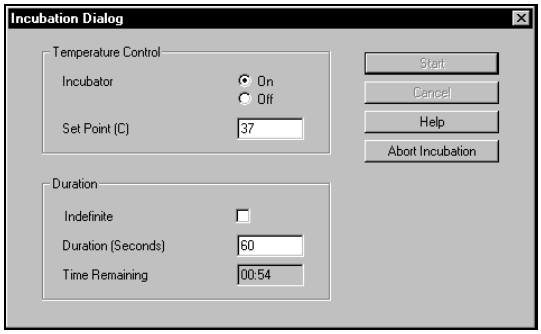


  Click the **Override** button to begin the incubation before the setpoint temperature is reached.

  Note: The acceptable temperature range for plate reading for most readers is +/- 2°C of the setpoint temperature. Because of this, KCjunior may provide the "Place plate on carrier" indication when the reader temperature is within 2°C of the set point temperature. The incubator temperature will continue to rise and then stabilize at the set point temperature.

- When the setpoint temperature is reached, place the plate in the carrier, then click **OK.**

  The plate incubation will begin. KCjunior will display the **Time Remaining.**



  Click the **Abort Incubation** button to stop the plate incubation.

- When the incubation is complete, remove the plate from the carrier.

*Incubate a plate **later**.*

4. To turn the incubator **on** now and incubate a plate *later*:
   - Click the **On** radio button.
   - Enter the **Set Point** temperature.

     The valid range for most readers is from 20°C (or 4°C above room temperature, whichever is greater), to 50°C.

- Select **Indefinite** duration.
- Click **Set.**

  The **Incubation** dialog will close and the reader temperature will begin to increase or decrease appropriately.

*Turn the incubator **off**.*

5. To turn the incubator **off:**
   - Click the **Off** radio button.
   - Select **Indefinite** duration.
   - Click **Set.** The **Incubation** dialog will close and the reader temperature will begin to decrease to room temperature.

*Set a **timer**.*

6. To set a timer (EL312I and EL340I readers only):
   - Manually set the temperature on the EL312I or EL340I. Refer to the reader's user manual for instructions.
   - In KCjunior's Incubation dialog, enter the **Set Point** temperature (optional, for visual effect only).
   - Click the **Start** button to start the timer.
   - When the timer reaches 0, KCjunior will provide an indication to place the plate in the carrier.

Carrier Out/In

If the reader type specified under **Setup|Reader** supports computer-controlled operation of the plate carrier or door, the **Carrier Out** and **Carrier In** options are accessible in the Utilities Menu.

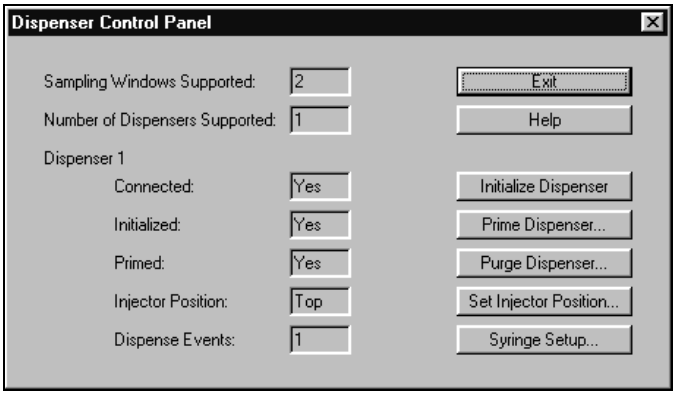
- Select **Carrier Out** to open the door or to extend the plate carrier.
- Select **Carrier In** to close the door or to retract the plate carrier.

Dispenser Control Panel

For the FLx800 reader with the optional reagent dispense capability, KCjunior provides a **Dispenser Control Panel.** The panel displays the current dispenser status and provides access to dispenser-specific functions such as prime and purge.

To access the Dispenser Control Panel:

1. Ensure that the reader is properly connected to the computer, and that all communication parameters are set correctly.
2. Ensure that FLx800 is selected as the Current Reader.
3. Select **Utilities|Dispenser Control Panel.**



- **Sampling Windows Supported:** The maximum number of unique sample measurement scenarios supported per well per kinetic read in a Dispense / Read protocol.
- **Number of Dispensers Supported:** The maximum number of dispensers that the reader currently supports.

For each dispenser, the following information is provided:

- **Connected:** Indicates whether or not the reader recognizes that the dispenser is connected.
- **Initialized:** Indicates whether or not the dispenser is initialized and ready for use.
- **Primed:** Indicates whether or not the prime operation has been run since the dispenser was last initialized.
- **Injector Position:** Displays the reader's current injector position setting (Top or Bottom).
- **Dispense Events:** The maximum number of dispenses supported per well in a Dispense / Read protocol.

Initializing the Dispenser

If the dispenser was connected before the reader was turned on, or if a system test was run, the dispenser should initialize automatically. If for any reason the dispenser does not initialize automatically, you can initialize it from KCjunior. This may be necessary if the dispenser was connected to the reader after the reader was turned on, or following the installation of a new syringe.

To initialize the dispenser:

1. Select **Utilities|Dispenser Control Panel.**
2. Click **Initialize Dispenser.** The dispenser's syringe drive will move to its home position and its sensors will be verified.

Priming the Dispenser

The dispenser's syringe and tubing must be fully primed with the desired fluid before plate reading can begin. In addition, the FLx800 operator's manual contains decontamination procedures that require the dispenser to be primed.

Important: A special priming plate (Bio-Tek part number 7142002) must be placed in the carrier prior to priming.

To prime the dispenser:

1. Fill the supply bottle with the desired fluid.
2. Place the priming plate on the carrier.
3. In KCjunior, select **Utilities|Dispenser Control Panel.**
4. Click **Prime Dispenser.**
5. Enter the desired **Prime Volume,** from 5 to 5000 ul.
6. Select a **Prime Dispense Rate** from 1 to 4.
   - **Note:** With each rate selection, the corresponding ul/sec value is displayed.
7. Click **Start Prime** to begin the prime.

Purging the Dispenser

KCjunior provides a utility for purging fluid from the dispenser by pumping the fluid in reverse, back into the supply bottle.

Important: The tip priming trough must be placed in the carrier prior to purging. During this process, the carrier is positioned so that the tip priming trough sits under the fluid injector head.

To purge the dispenser:

1. Ensure that the tip priming trough is in place on the carrier (see the FLx800 operator's manual for placement instructions).
2. In KCjunior, select **Utilities|Dispenser Control Panel.**
3. Click **Purge Dispenser.**
4. Enter the desired **Purge Volume,** from 5 to 5000 ul.
5. Click **Start Purge** to begin the purge.

Setting the Injector Position

The FLx800's fluid injector head can be positioned either next to the top probe or directly above the bottom probe. Refer to the FLx800 operator's manual for details.

If the fluid injector head is moved, the reader software must be reconfigured accordingly; this can only be done through KCjunior.

To configure the injector position setting:

1. In KCjunior, select **Utilities|Dispenser Control Panel.**
2. Click **Set Injector Position.**
3. Click the radio button associated with the **Injector Position** (next to top probe, or above bottom probe).
4. Click **Set Position.** The message "Injector position setting successfully completed" should appear.
5. Click **OK** to return to the Dispenser Control Panel. The Injector Position field will display the new position.

Syringe Setup

KCjunior provides access to special Syringe Setup functions for maintenance and calibration purposes.

- **Important:** Do not change the syringe position or calibrate the dispenser unless instructed to do so as part of installation, upgrade, or maintenance.

Moving the Syringe to its Maintenance Position

If the syringe needs to be installed or replaced, it must first be moved to its Maintenance Position. To do this from KCjunior:

1. Select **Utilities|Dispenser Control Panel.**
2. Click **Syringe Setup.**
3. Click **Move Syringe to Maintenance Position.**

   The syringe plunger will move to its furthest-from-home position. The syringe can then be disconnected from the drive bracket and unscrewed from the valve (see the FLx800 operator's or service manual for further details).

Diagnostics

The **Reader System Test** and the **Universal Plate Test** should be performed routinely to validate and document the consistency of a reader's optical and electronic performance, alignment, accuracy, reproducibility, and linearity.

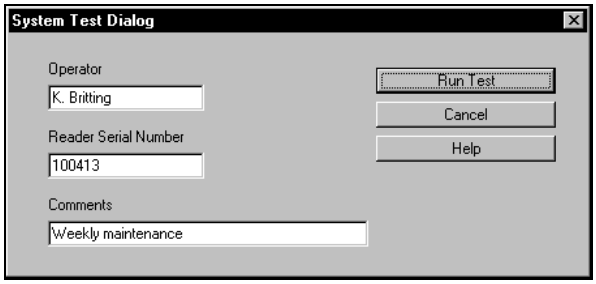
Reader System Test

The **Reader System Test** validates and documents the consistency of reader optical and electronic performance. For most readers, this test can be performed from within KCjunior.

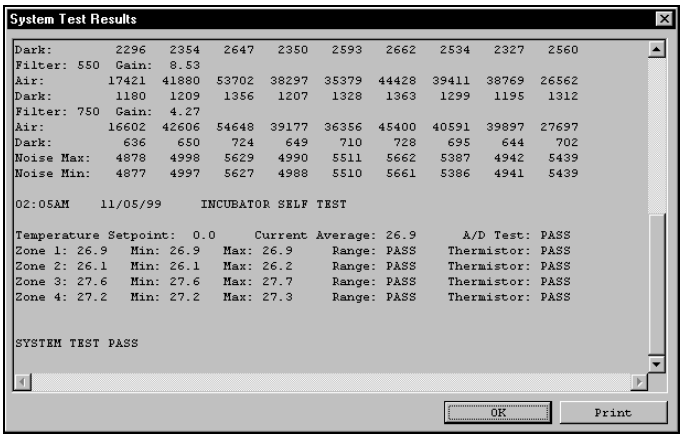
To perform a **Reader System Test:**

1. Ensure that the reader is properly connected to the computer, and that all communication parameters are set correctly.
2. From the **Utilities** menu, select **Diagnostics,** then **Reader System Test**.

   The **System Test** dialog appears:



3. Enter the **Reader Serial Number,** and an appropriate **Comment** if desired.
4. Click the **Run Test** button. KCjunior performs the test and displays the results. Sample test results are shown on the next page.



- Results should show SYSTEM TEST PASS.
- If the test **fails** and the reader begins "beeping", press the **Stop** button on the reader. The reader should then send an error code to KCjunior, resembling the following:

  SYSTEM TEST FAIL ERROR (0302) DETECTED

  Refer to the reader's user manual for a list of error codes and their potential causes.
- Click **Print** to print the results, or **OK** to close the results.

  Test results are automatically saved in the Results Database.

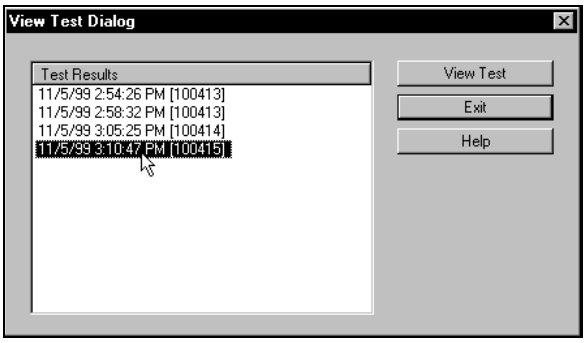
Reader System Test History

When a Reader System Test is complete, the test results are automatically saved to the Results Database. These test results can viewed at any time.

To view historical Reader System Test results:

1. Select **Utilities|Diagnostics|Reader System Test History.**

   The **View Test** dialog appears, listing the test results:



- The list is sorted by **date.** To switch between ascending and descending sort order, click the **Test Results** column header.

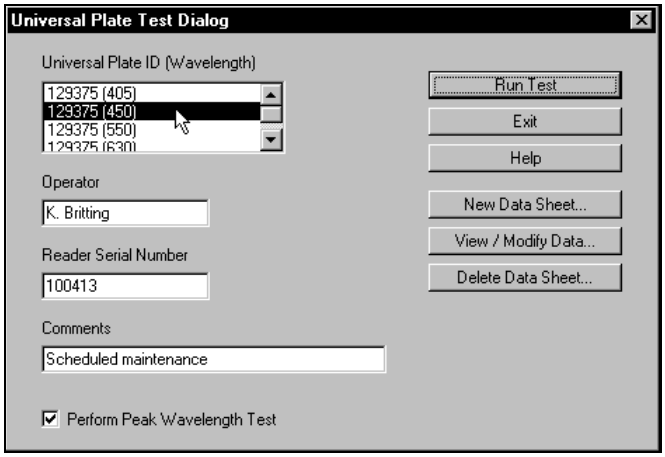
2. Highlight the desired test, then click the **View Test** button to view the results.
3. Click **OK** to close the results and return to the **View Test** dialog.
4. View another test, or click the **Exit** button to return to the main screen.

Universal Plate Test

The **Universal Plate Test** utilizes Bio-Tek's Universal Calibration Test Plate to validate and document the consistency of reader alignment, accuracy, reproducibility, and linearity. For most readers, this test can be performed from within KCjunior.

To perform the **Universal Plate Test:**

1. Ensure that the reader is properly connected to the computer, and that all communication parameters are set correctly.
2. The reader should be equipped with the necessary filters to perform the test. Refer to the calibration data table that came with the test plate to determine the required wavelengths.
3. Select **Utilities|Diagnostics|Universal Plate Test** to access the Universal Test Plate Dialog.
4. Examine the contents of the Universal Plate ID list box. The Universal Plate ID list box should contain a list of **data sheets** associated with the universal test plate. There should be one data sheet for each wavelength used for the test.



- If all of the required data sheets exist for the test plate, highlight the first **Plate ID (Wavelength)** to test, then skip ahead to step 5.
- If one or more data sheets need to be created, click the **New Data Sheet** button. See "Setting Up a New Data Sheet" for more information.

- If one or more data sheets needs to be viewed or modified, highlight a data sheet and click the **View/Modify** button.
- To delete a data sheet, highlight the desired item in the Universal Plate ID list, then click **Delete Data Sheet.** KCjunior will request confirmation before deletion.

5. If necessary, modify the **Operator** name.
6. Enter the **Reader Serial Number** and any **Comments.**
7. If supported by the reader, **Perform Peak Wavelength Test** is accessible. If this box is checked, the Peak Wavelength Test will be included in the universal plate test.
8. Click the **Run Test** button. The reader performs the test and KCjunior displays the results.
9. Click **Print** to print the results, or **OK** to close the results.

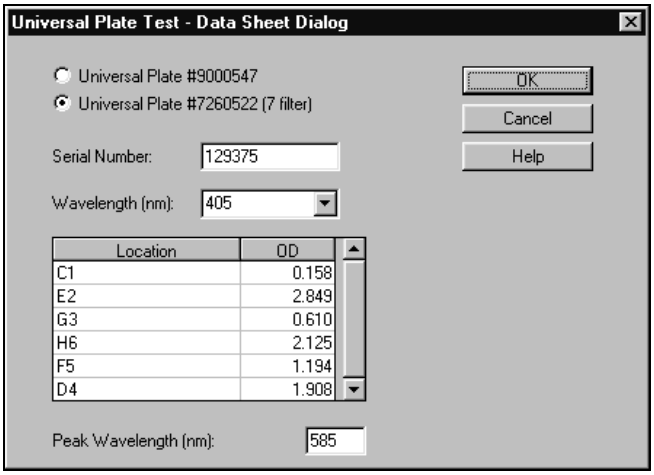
   Test results are automatically saved in the Results Database.
10. Repeat steps **4-8** for the remaining wavelengths.
11. Click the **Exit** button to return to the main screen.

Setting Up a New Data Sheet

A **data sheet** contains exact spectrophotometric calibration data associated with a neutral density filter at 1 of 8 possible wavelengths.

To create a **set** of data sheets (one for each wavelength), for use with the Universal Plate Test:

1. Click **New Data Sheet** from the Universal Plate Test dialog. The **Data Sheet** dialog appears:



2. Select a **Universal Plate.**
   - **#9000547** does not have a filter in the C6 location. It is the standard test plate that can be used on any Bio-Tek reader. If this plate is selected, the Peak Wavelength Test will not be performed.
   - **#7260522** has a filter in the C6 location, for the Peak Wavelength test. It is the test plate that is used on readers that support spectral scanning.
3. Enter the test plate's **Serial Number.**
4. Enter a **Wavelength,** or select one from the drop-down list.
5. Refer to the calibration data table that came with the test plate to determine the expected OD for each test well at the selected wavelength.

   For each **Location** (C1, D4, E2, F5, G3, H6), enter the **OD** associated with that well at the selected wavelength.

   For readers that support spectral scanning, a **Peak Wavelength** value is required. Enter the value in nanometers (nm).
6. Click **OK** to save the data sheet and return to the Universal Test Plate dialog.
7. Repeat the above steps to create a data sheet for each Universal Test Plate ID (Wavelength) to be tested.

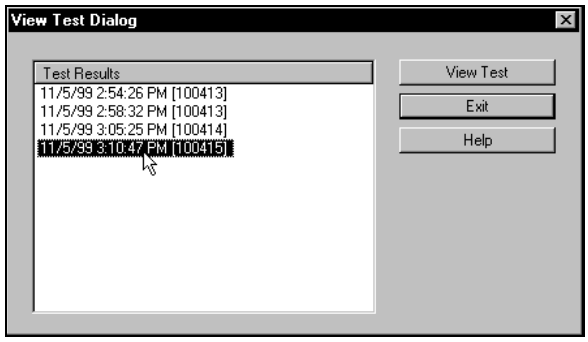
Universal Plate Test History

When a Universal Plate Test is complete, the test results are automatically saved in the Results Database. These test results can viewed at any time.

To view historical Universal Plate Test results:

1. Select **Utilities|Diagnostics|Universal Plate Test History.**

   The **View Test** dialog appears, listing all test results:



   - The list is sorted by **date.** To switch between ascending and descending order, click the **Test Results** column header.

2. Highlight the desired test, then click the **View Test** button to view the results.
3. Click **OK** to close the results and return to the **View Test** dialog.
4. View another test, or click the **Exit** button to return to the main screen.

Database Maintenance

Protocols, results records, stored curves, system test results, and universal plate test results can be copied into **archive databases** that can then be stored as a part of normal backup procedures.

During the archive process:

- Protocols are copied to an **Archive Protocol Database.**
- Results records, stored curves, system test results, and universal plate test results are copied to an **Archive Results Database.**

Archived or otherwise unnecessary protocols, results records, stored curves, stored transformations, system test results, and universal plate test results can be **deleted** from the Protocol and Results Databases currently in use.

A Note on Backup Procedures

It is strongly recommended that you **backup** (safely store) your protocol and results databases regularly. Here are some suggestions:

- Routinely copy the complete protocol and results databases from your hard drive to the network or to a floppy diskette. **Note:** Check to ensure that network files are automatically backed up.

  See "Pathnames" under *Setting Up the System* to find out where these databases are stored.

  - Enhance this suggestion by first copying certain protocols, results records, stored curves, and/or test results to **archive** protocol and results databases. Then copy the archive databases to the network or to a floppy diskette.

    Continue to review "Database Maintenance" for instructions.

- Instead of keeping your protocol and results databases on your hard drive, store them on the network.

  See "Sharing Protocols and Databases" under *Defining Protocols* and "Sharing Results Records and Databases" under *Reading Plates* for more information.

Creating Archive Databases

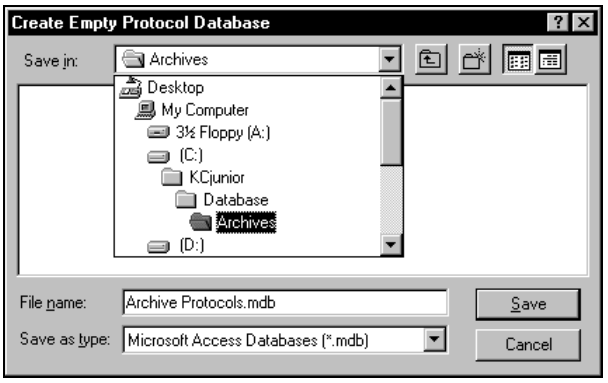
Before protocols can be archived, an **Archive Protocol Database** must be created. Before results records, stored curves, system test results, and universal plate test results can be archived, an **Archive Results Database** must be created.

During the archive process, selected items are copied from the *current* Results and Protocol Databases into the *archive* databases. Look under **Setup|Configuration|Pathnames** to see the names and locations of the currently configured Results and Protocol Databases.

To create an archive database:

1. Select **Utilities|Database Maintenance|Create Archive** (**Results** or **Protocol**) **Database.**

   The **Create Empty** (**Results** or **Protocol**) **Database** dialog appears.



2. In the **Save in** field select a location for the new database.
3. Enter an identifying **File name,** such as `ArchRslts.mdb` or `ArchProt.mdb`.
4. Click **Save** to create the database and return to the main screen.

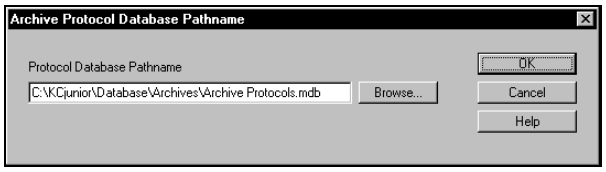
Archiving Results Records and Protocols

When results records and protocols are archived they are copied from the current Results and Protocol Databases, respectively, into the archive databases.

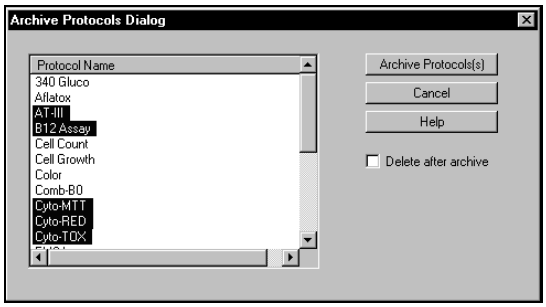
To archive results records and protocols:

1. Make sure that archive databases have been created.
2. Select **Utilities|Database Maintenance|Archive Results** or **Protocols.**

The **Archive Results** or **Protocol Database Pathname** dialog appears.



3. Enter the drive, directory, and file name of the archive database, or use the **Browse** button to locate this information.
4. Click **OK.** The **Archive Results** or **Archive Protocols** dialog appears, listing the contents of the current Results or Protocol Database.



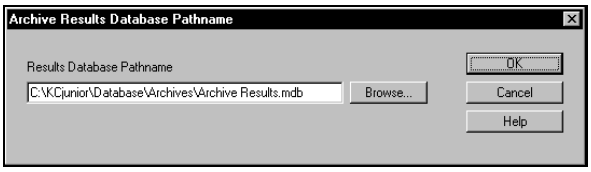
5. Select the results record(s) or protocol(s) to be archived:
   - If archiving results records, click the **Results** and **Creation Date** column headers to sort the records alphabetically or by date.
   - Use the mouse to select the items(s) to be archived. Press the **Shift** key while using the mouse to select contiguous items. Press the **Ctrl** key while using the mouse to select non-contiguous items.
   - Check **Delete after archive** to permanently delete the item(s) from the current database after being copied to the archive database.
6. Click the **Archive Results** or **Archive Protocol(s)** button. The selected items are copied to the archive database and then deleted from the current database (if **Delete after archive** is checked).
   - If a duplicate **Results Record** or **Protocol Name** is detected, KCjunior will **not** overwrite the results record or protocol.

Archiving Stored Curves

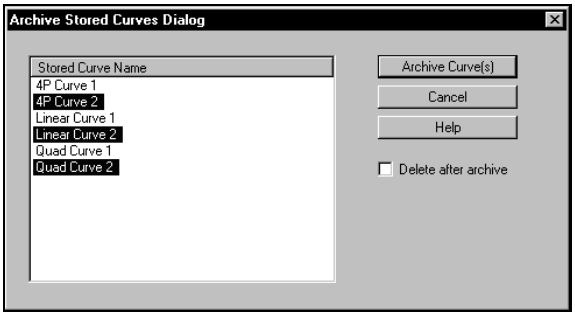
When stored curves are archived, they are copied from the current Results Database into the **Archive Results Database.**

To archive stored curves:

1. Make sure that an Archive Results Database has been created.
2. Select **Utilities|Database Maintenance|Archive Stored Curves.** The **Archive Results Database Pathname** dialog appears.



3. Enter the drive, directory, and file name of the archive database, or use the **Browse** button to locate this information.
4. Click **OK.** The **Archive Stored Curves** dialog appears, listing the curves stored in the current Results Database.



5. Select the curve(s) to be archived:
   - Use the mouse to select the items(s) to be archived. Press the **Shift** key while using the mouse to select contiguous items. Press the **Ctrl** key while using the mouse to select non-contiguous items.
   - Check **Delete after archive** to permanently delete the item(s) from the current database after being copied to the archive database.
6. Click the **Archive Curve(s)** button. The selected items are copied to the archive database and then deleted from the current database (if **Delete after archive** is checked).
   - If a duplicate **Curve Name** is detected, KCjunior does **not** overwrite the curve.

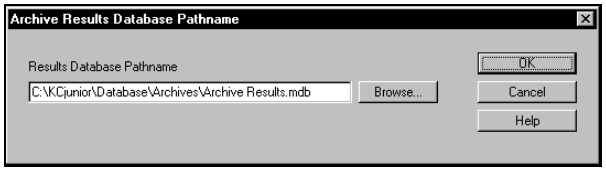
Archiving Test Results

When Reader System and Universal Plate Test Results are archived they are copied from the current Results Database into the **Archive Results Database.**

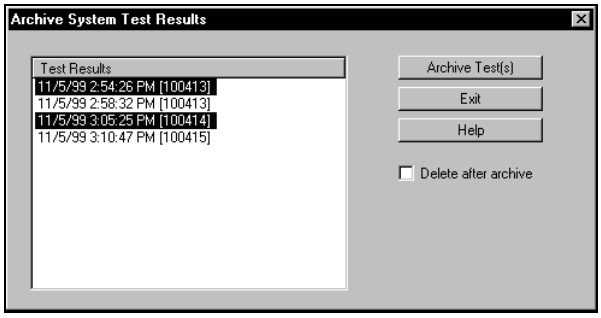
To archive test results:

1. Make sure that an Archive Results Database has been created.
2. Select **Utilities|Database Maintenance|Archive System Test Results** or **Universal Plate Test Results.**

   The **Archive Results Database Pathname** dialog appears.



3. Enter the drive, directory, and file name of the archive database, or use the **Browse** button to locate this information.
4. Click **OK.** The **Archive System Test Results** or **Archive Universal Plate Test Results** dialog appears, listing the test results stored in the current Results Database.



5. Select the test result(s) to be archived:
   - To switch between ascending and descending sort order, click the **Test Results** column header.
   - Use the mouse to select the items(s) to be archived. Press the **Shift** key while using the mouse to select contiguous items. Press the **Ctrl** key while using the mouse to select non-contiguous items.
   - Check **Delete after archive** to permanently delete the item(s) from the current database after being copied to the archive database.

6. Click the **Archive Test(s)** button. The selected items are copied to the Archive Results Database and then deleted from the current database (if **Delete after archive** is checked).
   - If a duplicate **Date/Time** is detected, KCjunior does not overwrite the existing test results.

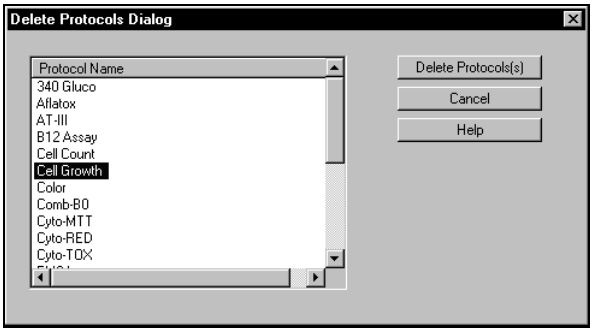
Deleting Results Records and Protocols

After results records and protocols have been safely archived, or if certain results records or protocols are no longer needed, they can be deleted from the current Results and Protocol Databases.

To delete results records and protocols:

1. Close any open records/protocols.
2. Select **Utilities|Database Maintenance|Delete Results** or **Delete Protocols.**

   The **Delete Results** or **Delete Protocols** dialog appears, listing all results records or protocols in the current database.



3. Select the results record(s) or protocol(s) to be deleted:
   - If deleting results records, click the **Results** and **Creation Date** column headers to sort the records alphabetically or by date.
   - Use the mouse to select the item(s) to be deleted. Press the **Shift** key while using the mouse to select contiguous items. Press the **Ctrl** key while using the mouse to select non-contiguous items.
4. Click the **Delete Results** or **Delete Protocol(s)** button. The selected items are permanently removed from the current database.

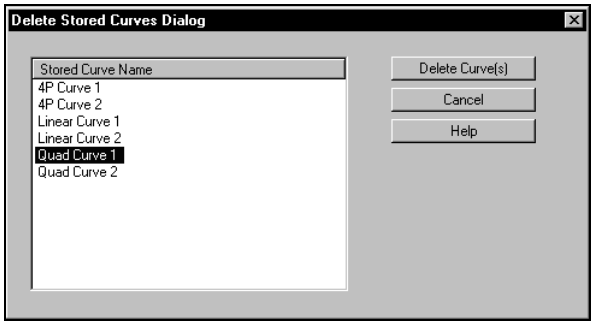
Deleting Stored Curves

After stored curves have been safely archived, or if certain curves are no longer needed, they can be deleted from the current Results Database.

To delete stored curves:

1. Close any open protocols.
2. Select **Utilities|Database Maintenance|Delete Stored Curves.**

   The **Delete Stored Curves** dialog appears, listing all curves stored in the current Results Database.



3. Select the curve(s) to be deleted.
   - Use the mouse to select the items(s) to be deleted. Press the **Shift** key while using the mouse to select contiguous curves. Press the **Ctrl** key while using the mouse to select non-contiguous curves.
4. Click the **Delete Curve(s)** button. The selected item(s) are permanently removed from the current Results Database.

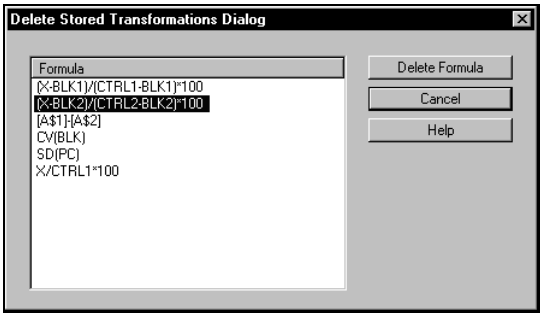
Deleting Stored Transformations

If certain stored transformations are no longer needed, they can be deleted from the current Protocol Database.

To delete stored transformations:

1. Close any open protocols.
2. Select **Utilities|Database Maintenance|Delete Stored Transformations.**

   The **Delete Stored Transformations** dialog appears, listing all formulas stored in the current Protocol Database.



3. Select the formula(s) to be deleted.
   - Use the mouse to select the items(s) to be deleted. Press the **Shift** key while using the mouse to select contiguous formulas. Press the **Ctrl** key while using the mouse to select non-contiguous formulas.
4. Click the **Delete Formula(s)** button. The selected item(s) are permanently removed from the current Protocol Database.

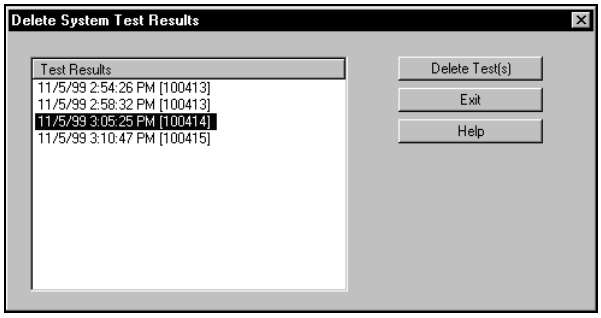
Deleting Test Results

After **System Test** and **Universal Plate Test** results have been safely archived, or if certain test results are no longer needed, they can be deleted from the current Results Database.

To delete stored System Test or Universal Plate Test results:

1. Close any open plates.
2. Select **Utilities|Database Maintenance|Delete System Test Results** or **Delete Universal Plate Test Results.**

   The **Delete System Test Results** or **Delete Universal Plate Test Results** dialog appears, listing all test results stored in the current Results Database.



3. Select the test(s) to be deleted.
   - Use the mouse to select the items(s) to be deleted. Press the **Shift** key while using the mouse to select contiguous tests. Press the **Ctrl** key while using the mouse to select non-contiguous tests.
4. Click the **Delete Test(s)** button. The selected item(s) are permanently removed from the current Results Database.

Troubleshooting

Overview

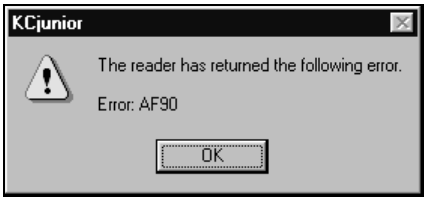
There are three categories of error and warning messages that may appear in KCjunior:

- **Reader** errors that occur during a plate read.
- **Communication** errors between the computer and the attached microplate reader.
- **Data reduction** comments and warnings.

This section describes every communication error and data reduction warning or comment that may occur while running KCjunior, and in many cases provides suggestions for fixing the error.

Reader Errors

If the reader experiences an error during a plate read, KCjunior may present a message resembling the following:



- If a reader error message is presented, note the **error code** (ex. AF90), then refer to the reader's Operator's Manual for more information. The manual should contain a table of possible error codes.
- KCjunior will attempt to save any data that was retrieved from the reader before the error occurred. The **Plate Status** will be updated with the reader error code. To check the Plate Status, select **Plate|Plate Information.**

Communication Errors

Reader Status Failure

KCjunior is unable to communicate with the attached reader.

Suggestions:

- Select **Setup|Reader,** then click **Test Communications.** KCjunior will provide appropriate messages for reconfiguring the communication parameters.
- Make sure that the proper **Reader Type** is selected under **Setup|Reader,** then run the reader system test.
- If the reader just experienced a failure, the reader system test must be run before plate reading can resume.

Reader Timeout Error

KCjunior received no response from the reader.

Suggestions:

- The reader should be on and at its main display when communicating with KCjunior.
- Try to perform a reader system test from within KCjunior.

If you cannot communicate with the reader, run the test from reader front panel. If the test fails, refer to the reader's user manual for assistance.

- If the error appeared while performing a kinetic read:

  Close all unnecessary programs that are running concurrently with KCjunior (see “System Requirements” in *Installing the Software* for more information).

  If the error persists with a fast kinetic read, you may need to lengthen the kinetic interval.

Serial Read Error

An error occurred when KCjunior attempted to communicate with the reader.

Suggestions:

- Make sure that the reader is on and ready to accept serial commands.
- Check to see if the correct serial cable is being used, and that it is properly connected to the PC and the reader.
- Check that the communication settings under **Setup|Reader** are correct for this reader.

  The communication parameters can be tested from within the **Setup|Reader** dialog.

Serial Write Error

An error occurred when KCjunior attempted to communicate with the reader.

Suggestions:

- Make sure that the reader is on and ready to accept serial commands.
- Check that the communication settings under **Setup|Reader** are correct for this reader.

  The communication parameters can be tested from within the **Setup|Reader** dialog.

Comments and Warnings

?????? indicates a calculation could not be performed

If a well contains **??????,** one of the following occurred during data reduction:

- The well references another well with a status of:
  - Well Read Error
  - OUT
  - Masked
  - Divide by Zero
  - Well Failure
- A calculation within or involving the well:
  - attempted an invalid logarithmic function.
  - is syntactically invalid.
  - references an invalid location.
  - references an invalid Well ID.
  - attempted a Standard Deviation on a well group with an insufficient sample population.
  - attempted a Coefficient of Variation on a well group with an insufficient sample population.
- The well is in a blank sector that contains blanks but none of them are valid.
- Cutoffs could not be evaluated for the well.
- A concentration had no valid solutions on the generated curve.
- The curve could not be generated.

[ ] indicates a well was set to the maximum valid value

The Raw Data value measured for this well exceeded the upper limit of the valid data range that was defined in the protocol. KCjunior is currently configured to use this altered value in further calculations.

Suggestions:

- To extend the upper limit of this range, or to exclude this and all out-of-range wells from further calculations:
  1. Click to access the **General Information** dialog.

2. Do one of the following:

   Set the **Raw Data** or **Blank Limits Maximum Value** to the desired OD, RFU, or RLU.

   Select **Discard Out-of-Range Values** for Raw Data Limits or Blank Limits, as appropriate. All out-of-range wells will be reported as **OUT.**

3. Click **OK** to accept the change.

[ ] indicates a well was set to the minimum valid value

The Raw Data value measured for this well fell below the lower limit of the valid data range that was defined in the protocol. KCjunior is currently configured to use this altered value in further calculations.

Suggestions:

- To extend the lower limit of this range, or to exclude this and all out-of-range wells from further calculations:

  1. Click to access the **General Information** dialog.
  2. Do one of the following:

     Set the **Raw Data** or **Blank Limits Minimum Value** to the desired OD, RFU, or RLU.

     Select **Discard Out-of-Range Values** for Raw Data Limits or Blank Limits, as appropriate. All out-of-range wells will be reported as **OUT.**

  3. Click **OK** to accept the change.

( ) indicates an extrapolated concentration

The concentration for this well was calculated through **extrapolation.**

If **Extrapolate** is enabled in the protocol, the curve is extended outside the total range of the standards in order to calculate a concentration for an OD, RFU, or RLU value beyond the highest and lowest standard values.

Suggestion:

- To disable Extrapolation:

  1. Click to modify the protocol.
  2. Click the **Curve** tab.
  3. Deselect **Extrapolate.**
  4. Click **OK** to accept the change and recalculate the results.

{ } indicates ambiguous concentrations

Two or more concentrations were calculated for the Y-value in this well.

- KCjunior automatically reports the **largest** concentration.

***** indicates a well read error

A well read error may be caused by one of the following:

- The reader determined that the OD, RFU, or RLU in this well was significantly out-of-range, then set the error status before sending data to KCjunior.
- If plate data was read from an Omni-System absorbance file, the absorbance file may contain an invalid status (1, 2, 89, or 99) for that well, indicating an error during plate processing or data reduction.

A memory allocation error has occurred.

KCjunior was unable to access system memory.

Suggestions:

- This error may occur if so many programs are open at once that system memory is overly consumed. Close any unnecessary programs.
- Make sure that your computer meets the minimum system requirements, listed on page 2-2 in “Installing the Software”.

BTI shared library could not be found

The file `BTI_DLL.DLL` is missing from the Windows\System directory.

Suggestions:

- Search your hard drive to see if the file has been moved. If you find the file, move it back to the Windows\System directory.
- If you cannot find the file, reinstall KCjunior.

Cutoff X could not be calculated

A cutoff formula defined in the protocol could not be calculated.

Suggestions:

- Check the **Cutoff** dialog in the protocol to ensure that cutoffs are defined correctly.
- If a cutoff references a well ID (ex. “PC”), this error may indicate that all replicates of the well ID are invalid.

Cutoff X is not greater than the previous cutoff

Two or more cutoffs are defined in the protocol and they are not in ascending order.

Suggestions:

- Check the **Cutoff** dialog in the protocol to ensure that cutoffs are defined correctly.
- If a cutoff references a well ID (ex. “STD02”), this error may indicate that the actual mean value for the well ID greatly varies from its expected value.

DIV/0

DIV/0 represents an attempt to divide by zero.

Suggestion:

- This error is most likely to appear in the Statistics dialog after an unsuccessful attempt at a **CV%** calculation. Click the **Statistics** button to view the results. Any DIV/0 warnings will appear in the CV% column.

Error during auto sensitivity

This message indicates a failure during the auto sensitivity adjustment process. The most likely reason is an error during the calculation of the signal-to-noise ratio.

Review the Auto Sensitivity selections in the protocol:

- Ensure that the Low, Test, and High Wells are assigned correctly.
  - The Low Well(s) are usually blank wells, buffer wells, zero standards, or negative controls.
  - The Test Well(s) are usually low standards or low positive controls.
  - The High Well(s) are usually the highest standards or high positive controls.
- The Base Value should be greater than zero. The default setting is 100.
- If the High Value is set too low, the Low, Test, and/or High Wells can overrange before two valid signal-to-noise ratios can be calculated.

Grayed numbers indicate a suppressed standard

If, in the Results displays, a standard well contains a "grayed" value, this indicates that the standard has been suppressed.

- To **restore** the standard, click Standard Curve, click Edit Stds…, then change the Status of the desired well.

Illegal log function attempted on a standard

This error typically results during curve generation if the Y-Scale value is set to Log and the OD, RFU, or RLU value for a standard or dilution well is 0.000.

- For **titer** curves, the warning will report the Sample ID(s) experiencing the error.

Insufficient valid standards for this curve type

To generate a successful curve, the selected curve method requires more standards than are currently valid on the plate.

Suggestions:

- Check the Raw Data values for the standards. If one or more standards is reported as **OUT,** this means that the OD, RFU, or RLU value fell outside the minimum/maximum data range defined in the protocol and is excluded from further calculations.
- If you just edited one or more standard outliers, there may now be too few remaining valid standards.
- The minimum number of required valid standards for each curve method are:

  Linear and Point-to-Point (2)

  Quadratic and Cubic-Spline (3)

  Cubic, 2 Parameter and 4 Parameter (4).

No valid blanks in sector X

All blank wells in one or more sectors in the plate are invalid.

Suggestion:

- If all blank wells in a sector are reported as **OUT,** the Raw Data values for these wells exceeded the minimum/maximum data range defined in the protocol.

  Click to open the General Information dialog to see the current Raw Data Limits and Blank Data minimum and maximum settings.

OUT indicates a well exceeded the min/max range

The Raw Data value measured for this well fell outside the valid data range as determined by the reader or as defined in the protocol. Out-of-range values are currently being **excluded** from further calculations.

Suggestion:

- To **include** out-of-range wells in further calculations:

  1. If a protocol is open, click to access the **General Information** dialog.

     If no protocol is open, select **Protocol|New Protocol.** The General Information dialog will appear.

  2. Alter the Raw Data Limits and/or Blank Limits **Minimum** and **Maximum** values, if necessary.
  3. Deselect **Discard Out-of-Range Values** for Raw Data Limits or Blank Limits, as appropriate.
  4. Click **OK** to accept the change(s).

     All wells that were previously reported as OUT will be set to the **minimum/maximum values** as appropriate and displayed in brackets. These altered values will now be used in further calculations.

The Pathlengths could not be generated

This warning will appear if, during the read, the **Abort Read** button was clicked to halt the read in progress before the necessary pathlength correction wavelengths were read.

Plate was aborted during read

This warning will appear if, during a read, the **Abort Read** button was clicked to halt the read in progress.

- KCjunior will attempt to perform data reduction based on any data retrieved up to the time the button was clicked.

Reader control error occurred during read

This warning will appear if, during a plate read the reader experiences a failure and is unable to respond to the computer.

- The reader control error may appear if there is a serial communication failure during the plate read.
- If the reader control error appears, try the following:
  - Turn off the reader.
  - Ensure that the serial cable is properly connected.

- Close KCjunior then reboot the computer.
- Turn the reader on. Run a system test.
- Launch KCjunior.
- Under **Setup|Reader,** check the communication parameters, then click **Test Communications.** If the test is successful, try re-reading the plate.

- Refer to the reader's Operator's Manual for information on specific reader failures and corrective action.

Strikethrough indicates a masked well

A well with a strikethrough ~~2.577~~ indicates that the well is "masked", or temporarily excluded from calculations.

To **unmask** the well:

1. Highlight the well and click the **right** mouse button.
2. Select ✓ **Mask Well.**
3. KCjunior will automatically recalculate the results.

Stored curve does not exist

The protocol references a stored curve that does not exist in the current Results Database.

Suggestions:

- Choose to modify the protocol then click the **Curve** tab. Select a different **Stored Curve.**
- Open the **System|Configuration|Pathnames** dialog. Change the **Results Database Path** name to access a different database.

Too many standards for curve type

Too many standards or unique dilutions (titer assay) were defined for the curve type.

- For example, the 2P (Logit-Log) and 4P curve types allow a maximum number of **24** standard groups.

Unable to generate curve

The curve could not be generated. Possible reasons include:

- No solution is available for the given set of points.
- If this is a titer assay, there may be insufficient dilutions defined for the selected curve method.
- There are insufficient valid standards due to OUT, masked, suppressed, or failed (*.***) well conditions, or other data reduction failures.
- The concentrations are all equal, resulting in a slope of infinity.

Unable to initialize curve standards

If this is a titer assay, **all** replicates within a sample group are currently invalid.

- Invalid notations include:
  - OUT (well is out-of-range)
  - *.*** (well status error)
  - Strikethrough ~~2.765~~ (well is masked).

Appendix A

Sample Reports

This section contains sample KCjunior reports. Reports are selected within a protocol, under the **Reports** tab. See “Reports” in *Defining Protocols* for more information.

Sample Report
PROTOCOL/TEMPLATE REPORT

Protocol ID: Sample Protocol #1
Plate ID: Sample Plate #1

Plate Read Time: 4/23/99 8:47:27 AM
Temperature: 37.0 C

General Information

Protocol Description
This report contains sample data.
You can enter a Protocol Description using up to 1024 alphanumeric characters.

Raw Data Limits:	-3.000 to 3.000	☒ Discard Out-of-Range Values
Blank Data Limits:	-3.000 to 3.000	☒ Discard Out-of-Range Values
Decimal Points:	3	☐ Scientific Notation (Raw Data / Blanks)
		☐ Scientific Notation (Transforms / Concentrations)
Blank Method:	Full	

Read Method Information

Chemistry:	Absorbance
Read Method:	Endpoint
Primary Wavelength:	405 nm
Reference Wavelength:	n/a
Geometry:	8x12
Delay Before Read:	10 secs
Incubation:	37 C
Shaking:	Before first read, low intensity, 30 secs

Curve Information

Curve Method:	4 Parameter	
Graph Name:	Cytotoxicity	
Y Axis:	Linear	Absorbance
X Axis:	Linear	mmol
Average Standards:	Yes	
Extrapolate:	Yes	
Interpolation Value:	0.100	

Transformations

Loc	Transformation Formula
A1	(([A$2]-[A$12])/2)+[A$12]
B1	(([B$2]-[B$12])/2)+[B$12]
C1	(([C$2]-[C$12])/2)+[C$12]
D1	(([D$2]-[D$12])/2)+[D$12]
E1	(([E$2]-[E$12])/2)+[E$12]
F1	(([F$2]-[F$12])/2)+[F$12]
G1	(([G$2]-[G$12])/2)+[G$12]
H1	(([H$2]-[H$12])/2)+[H$12]

Plate Formula

X*100

Cutoff Information

Cutoff	Label
	< .05
STD02	< .2
STD04	< 1
STD06	< 3
STD08	>= 3

Protocol Report for an absorbance assay, showing Formula information.

Sample Report
PROTOCOL/TEMPLATE REPORT

Protocol ID: Sample Fluoro #1
Plate ID: Sample Fluoro #1

Plate Read Time: 11/18/99 4:08:26 PM
Temperature: n/a

General Information

Raw Data Limits:	0 to 99999	☒ Discard Out-of-Range Values
Blank Data Limits:	0 to 99999	☒ Discard Out-of-Range Values
Decimal Points:	3	☐ Scientific Notation (Raw Data / Blanks)
		☐ Scientific Notation (Transforms / Concentrations)
Blank Method:	Full	

Read Method Information

Chemistry:	Fluorescence
Read Method:	Endpoint
Filter Set 1:	Excite: 485/20 Emit: 530/20, Bottom, Sensitivity: 75
Filter Set 2:	Excite: 485/20 Emit: 530/20, Bottom, Sensitivity: 100
Filter Set 3:	Excite: 485/20 Emit: 530/20, Bottom, Sensitivity: 125
Filter Set 4:	Excite: 485/20 Emit: 530/20, Top, Sensitivity: 75
Filter Set 5:	Excite: 485/20 Emit: 530/20, Top, Sensitivity: 100
Filter Set 6:	Excite: 485/20 Emit: 530/20, Top, Sensitivity: 125
	☐ Eject between filter set reads
Geometry:	8x12 Reading Range: A1-H12
Incubation:	n/a
Shaking:	n/a

Template

	01	02	03	04	05	06	07	08	09	10	11	12	
A	SMP01	SMP02	SMP03	SMP04	SMP05	SMP06	SMP07	SMP08	SMP09	SMP10	SMP11	SMP12	Well ID
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	Conc/Dil
B	SMP01	SMP02	SMP03	SMP04	SMP05	SMP06	SMP07	SMP08	SMP09	SMP10	SMP11	SMP12	Well ID
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	Conc/Dil
C	SMP01	SMP02	SMP03	SMP04	SMP05	SMP06	SMP07	SMP08	SMP09	SMP10	SMP11	SMP12	Well ID
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	Conc/Dil
D	SMP01	SMP02	SMP03	SMP04	SMP05	SMP06	SMP07	SMP08	SMP09	SMP10	SMP11	SMP12	Well ID
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	Conc/Dil
E	SMP01	SMP02	SMP03	SMP04	SMP05	SMP06	SMP07	SMP08	SMP09	SMP10	SMP11	SMP12	Well ID
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	Conc/Dil
F	SMP01	SMP02	SMP03	SMP04	SMP05	SMP06	SMP07	SMP08	SMP09	SMP10	SMP11	SMP12	Well ID
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	Conc/Dil
G	SMP01	SMP02	SMP03	SMP04	SMP05	SMP06	SMP07	SMP08	SMP09	SMP10	SMP11	SMP12	Well ID
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	Conc/Dil
H	SMP01	SMP02	SMP03	SMP04	SMP05	SMP06	SMP07	SMP08	SMP09	SMP10	SMP11	SMP12	Well ID
	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	Conc/Dil

Protocol Report for a fluorescence assay, showing the Template information.

Sample Report
RAW DATA REPORT

Protocol ID: Sample Protocol #2
Plate ID: Sample Plate #2

Plate Read Time: 4/23/99 9:03:33 AM
Temperature: 36.9 C

Warning Messages

An 'M' in front of a value indicates a masked well
OUT indicates a well exceeded the min/max range

	01	02	03	04	05	06	07	08	09	10	11	12	
A	0.456	0.471	0.668	0.735	1.505	0.843	0.633	0.634	0.921	2.122	0.699	2.054	Raw Data
	-0.008	0.008	0.205	0.271	1.042	0.379	0.169	0.170	0.458	1.658	0.236	1.590	Blkd Data
B	0.526	0.673	0.994	0.901	1.072	0.746	1.417	1.862	0.783	1.618	0.689	1.217	Raw Data
	0.063	0.210	0.530	0.438	0.609	0.282	0.954	1.399	0.320	1.155	0.226	0.754	Blkd Data
C	0.744	0.744	1.239	0.859	0.755	1.420	0.906	1.970	0.819	0.898	1.854	0.891	Raw Data
	0.280	0.280	0.776	0.395	0.291	0.956	0.443	1.507	0.356	0.435	1.391	0.427	Blkd Data
D	M2.455	1.238	0.790	0.981	1.048	0.740	0.821	0.780	1.201	1.054	0.977	0.926	Raw Data
		0.774	0.327	0.517	0.585	0.276	0.358	0.317	0.738	0.591	0.513	0.463	Blkd Data
E	1.198	1.319	0.892	1.458	1.295	0.803	0.855	0.809	0.817	0.872	1.051	1.300	Raw Data
	0.734	0.855	0.428	0.994	0.831	0.340	0.391	0.346	0.354	0.408	0.587	0.837	Blkd Data
F	0.641	0.737	0.943	0.881	0.925	1.255	0.685	0.725	0.643	0.823	0.816	0.934	Raw Data
	0.177	0.273	0.480	0.417	0.462	0.792	0.222	0.261	0.179	0.360	0.353	0.471	Blkd Data
G	OUT	2.449	0.888	1.067	1.655	2.395	0.789	1.133	1.820	1.821	1.842	1.063	Raw Data
		1.985	0.424	0.603	1.192	1.932	0.326	0.669	1.357	1.357	1.379	0.599	Blkd Data
H	1.746	1.638	1.072	1.824	1.581	0.947	0.897	0.798	2.143	2.085	2.499	1.764	Raw Data
	1.283	1.175	0.609	1.361	1.117	0.484	0.434	0.335	1.680	1.621	2.035	1.301	Blkd Data

11/18/99 4:10:11 PM

Page 1 of 1

Raw Data Report.

Sample Report (6x8 well format)
MAXIMUM SLOPE REPORT

Protocol ID: Sample Protocol #3
Plate ID: Sample Plate #3

Plate Read Time: 04/22/99 03:22:28 PM
Temperature: n/a

	01	02	03	04	05	06	07	08	
A	3150.00	3415.38	2884.62	4015.38	3807.69	3230.77	2769.23	3230.77	Max Slope
B	3646.15	2884.62	4592.31	3807.69	4015.38	3461.54	3461.54	2769.23	Max Slope
C	3876.92	4500.00	3410.77	2838.46	3646.15	3692.31	3692.31	3000.00	Max Slope
D	4546.15	3207.69	2307.69	3046.15	2861.54	2769.23	4153.85	3230.77	Max Slope
E	2630.77	3253.85	2838.46	4015.38	3392.31	3461.54	3923.08	3461.54	Max Slope
F	2353.85	2330.77	3807.69	2884.62	4613.08	2838.46	3461.54	4384.62	Max Slope

04/23/99 09:13:57 AM

Page 1 of 1

Maximum Slope Report (6x8 Plate Geometry).

Sample Report
FORMULA REPORT

Protocol ID: Sample Protocol #1
Plate ID: Sample Plate #1

Plate Read Time: 04/23/99 08:47:27 AM
Temperature: 37.0 C

	01	02	03	04	05	06	07	08	09	10	11	12	
A	1.275	0.474	0.668	0.735	1.506	0.844	0.635	0.636	0.924	2.126	0.702	2.075	Transform
	45.800	47.400	66.800	73.500	150.600	84.400	63.500	63.600	92.400	212.600	70.200	207.500	Plt Trans
B	0.949	0.675	0.995	0.902	1.071	0.747	1.420	1.864	0.786	1.628	0.690	1.223	Transform
	52.800	67.500	99.500	90.200	107.100	74.700	142.000	186.400	78.600	162.800	69.000	122.300	Plt Trans
C	0.821	0.746	1.242	0.860	0.756	1.424	0.909	1.976	0.821	0.901	1.864	0.895	Transform
	74.800	74.600	124.200	86.000	75.600	142.400	90.900	197.600	82.100	90.100	186.400	89.500	Plt Trans
D	1.085	1.240	0.790	0.982	1.048	0.741	0.823	0.781	1.206	1.059	0.980	0.930	Transform
	245.700	124.000	79.000	98.200	104.800	74.100	82.300	78.100	120.600	105.900	98.000	93.000	Plt Trans
E	1.313	1.320	0.894	1.460	1.298	0.802	0.857	0.813	0.819	0.875	1.058	1.305	Transform
	119.900	132.000	89.400	146.000	129.800	80.200	85.700	81.300	81.900	87.500	105.800	130.500	Plt Trans
F	0.838	0.737	0.944	0.882	0.925	1.257	0.687	0.727	0.646	0.828	0.816	0.938	Transform
	64.200	73.700	94.400	88.200	92.500	125.700	68.700	72.700	64.600	82.800	81.600	93.800	Plt Trans
G	1.760	2.452	0.890	1.069	1.656	2.398	0.790	1.137	1.822	1.825	1.851	1.067	Transform
	271.900	245.200	89.000	106.900	165.600	239.800	79.000	113.700	182.200	182.500	185.100	106.700	Plt Trans
H	1.707	1.638	1.074	1.828	1.581	0.949	0.901	0.801	2.147	2.101	2.518	1.775	Transform
	174.900	163.800	107.400	182.800	158.100	94.900	90.100	80.100	214.700	210.100	251.800	177.500	Plt Trans

Transformations

Loc	Transformation Formula
A1	(([A$2]-[A$12])/2)+[A$12]
B1	(([B$2]-[B$12])/2)+[B$12]
C1	(([C$2]-[C$12])/2)+[C$12]
D1	(([D$2]-[D$12])/2)+[D$12]
E1	(([E$2]-[E$12])/2)+[E$12]
F1	(([F$2]-[F$12])/2)+[F$12]
G1	(([G$2]-[G$12])/2)+[G$12]
H1	(([H$2]-[H$12])/2)+[H$12]

Plate Formula

X*100

04/23/99 09:15:20 AM

Page 1 of 1

Formula Report with Transformation and Plate Formula information.

Sample Report
COLUMN REPORT

Protocol ID: Sample Protocol #4
Plate ID: Sample Results #4

Plate Read Time: 11/30/2000 8:23:59 AM
Temperature: n/a

Warning Messages

OUT indicates a well exceeded the min/max range
?????? indicates a calculation could not be performed
An 'S' in front of a value indicates a suppressed standard
( ) indicates an extrapolated concentration

Standards

Well ID	Loc	Concentration	Raw Data	Calc'd Conc	Std Dev	CV%	Label
STD01	A1	0.000	1.312	17.955			NEG
STD01	A2	0.000	1.325	15.460			NEG
	[AVE]		1.319	16.707	1.764	10.556	NEG
STD02	B1	25.000	1.287	22.377			NEG
STD02	B2	25.000	1.244	29.334			EQV
	[AVE]		1.265	25.856	4.919	19.025	EQV
STD03	C1	50.000	1.036	60.627			EQV
STD03	C2	50.000	1.205	35.283			EQV
	[AVE]		1.121	47.955	17.920	37.369	EQV
STD04	D1	100.000	S3.598	??????			??????
STD04	D2	100.000	0.781	107.652			EQV
	[AVE]		2.189	107.652			EQV
STD05	E1	200.000	0.527	192.560			EQV
STD05	E2	200.000	0.526	193.073			POS
	[AVE]		0.526	192.816	0.363	0.188	EQV
STD06	F1	400.000	0.322	(414.352)			POS
STD06	F2	400.000	0.324	(409.482)			POS
	[AVE]		0.323	411.917	3.443	0.836	POS

Controls/Blanks/Samples

Well ID/Sample ID	Loc	Dilution	Raw Data	Calc'd Conc	Std Dev	CV%	Label
0100	G1	1.000	0.512	200.524			POS
0100	G2	1.000	0.484	217.068			POS
	[AVE]		0.498	208.796	11.699	5.603	POS
0200	H1	1.000	1.201	35.883			EQV
0200	H2	1.000	1.192	37.227			EQV
	[AVE]		1.196	36.555	0.951	2.601	EQV
0300	A3	1.000	1.238	30.265			EQV
0300	A4	1.000	1.239	30.110			EQV
	[AVE]		1.239	30.188	0.109	0.362	EQV
0400	B3	1.000	1.110	49.383			EQV
0400	B4	1.000	1.097	51.325			EQV
	[AVE]		1.103	50.354	1.373	2.727	EQV
0500	C3	1.000	1.255	27.607			EQV
0500	C4	1.000	1.209	34.682			EQV
	[AVE]		1.232	31.145	5.003	16.064	EQV
0600	D3	1.000	1.320	16.440			NEG
0600	D4	1.000	1.298	20.480			NEG
	[AVE]		1.309	18.460	2.856	15.473	NEG

Column Report, page 1 of 2.

COLUMN REPORT (cont.)

Protocol ID: Sample Protocol #4
Plate ID: Sample Results #4

Plate Read Time: 11/30/2000 8:23:59 AM
Temperature: n/a

Well ID/Sample ID	Loc	Dilution	Raw Data	Calc'd Conc	Std Dev	CV%	Label
0700	E3	1.000	0.141	??????			??????
0700	E4	1.000	0.275	(588.724)			POS
	[AVE]		0.208	(588.724)			POS
0800	F3	1.000	0.280	(561.934)			POS
0800	F4	1.000	OUT				
	[AVE]		0.280	(561.934)			POS
0900	G3	1.000	1.117	48.341			EQV
0900	G4	1.000	1.076	54.487			EQV
	[AVE]		1.097	51.414	4.346	8.453	EQV
1000	H3	1.000	0.461	232.656			POS
1000	H4	1.000	0.432	255.652			POS
	[AVE]		0.447	244.154	16.261	6.660	POS

Standard Curve Information

Curve Type: 4 Parameter (y = (A - D) / (1 + (x/C)^B) + D)
Parameters: A=1.3753 B=1.6163 C=105.3927 D=0.2068, R-Square=0.9862
Interpolation Value: 1.000
Interpolation Result: 66.327

11/30/2000 9:04:40 AM

Page 2 of 2

Column Report, page 2 of 2.

COLUMN REPORT (cont.)

Protocol ID: SAMPLE Cytotoxicity
Plate ID: SAMPLE Cytotoxicity

Plate Read Time: 1/9/1992 12:07:59 PM
Temperature: n/a

Well ID/Sample ID	Loc	Dilution	Transform	Calc'd Conc	Std Dev	CV%	Label
SMP03	A9	512.000	72.934	497.051			
SMP03	A10	512.000	71.313	(592.347)			
SMP03	A11	512.000	74.878	401.605			
SMP03	B9	256.000	80.173	220.477			
SMP03	B10	256.000	81.037	199.265			
SMP03	B11	256.000	78.984	252.965			
SMP03	C9	128.000	84.819	126.290			
SMP03	C10	128.000	84.927	124.610			
SMP03	C11	128.000	84.495	131.449			
SMP03	D9	64.000	91.194	54.889			
SMP03	D10	64.000	89.897	65.548			
SMP03	D11	64.000	88.601	77.930			
SMP03	E9	32.000	95.300	30.228			
SMP03	E10	32.000	93.355	40.385			
SMP03	E11	32.000	93.247	41.024			
SMP03	F9	16.000	96.488	25.141			
SMP03	F10	16.000	97.569	21.151			
SMP03	F11	16.000	98.325	18.679			
SMP03	G9	8.000	103.728	6.974			
SMP03	G10	8.000	104.052	6.530			
SMP03	G11	8.000	105.457	4.855			
SMP03	H9	4.000	110.427	(1.405)			
SMP03	H10	4.000	105.240	5.088			
SMP03	H11	4.000	107.509	(3.033)			
	[AVE]		91.415	53.231	163.805	133.314	

Titer Results Information

Curve Type: 4 Parameter (y = (A - D) / (1 + (x/C)^B) + D)
Interpolation Value: 50.000

Specimen ID	Curve Parameters	R-Square	Interpolation
SMP01	A=112.6209 B=1.0524 C=43.2727 D=0.9677	0.9947	54.596
SMP02	A=121.0374 B=0.6725 C=150.6673 D=-28.6119	0.9986	129.595
SMP03	A=123.2737 B=0.2989 C=2239.4425 D=-6.0131	0.9800	(5500.44)

11/30/2000 9:33:26 AM

Page 4 of 4

Column Report showing Titer Results Information.

Sample Report
MATRIX REPORT

Protocol ID: Sample Protocol #6
Plate ID: Sample Plate #6

Plate Read Time: 06/23/98 01:10:36 PM
Temperature: n/a

Warning Messages

OUT indicates a well exceeded OD range
An 'S' in front of a value indicates a suppressed standard
( ) indicates an extrapolated concentration

	01	02	03	04	05	06	07	08	09	10	11	12	
	SMP01	CTRL	STD01	STD02	STD03	STD04	STD05	STD06	STD07	STD08	STD09	STD10	Well ID
	0.000	0.213	0.310	0.173	0.204	0.179	S0.170	0.150	0.118	0.091	0.040	0.010	Raw Data
A	0.111	0.213	0.310	0.173	0.204	0.179	S0.170	0.150	0.118	0.091	0.040	0.010	Transform
	1.000	1.000	0.001	0.050	0.100	0.200	0.500	1.000	2.000	3.000	5.000	10.000	Conc/Dil
	1.807	0.164	0.004	0.459	0.210	0.397	0.494	0.785	1.577	2.755	7.529	(13.388)	Calc Conc
	SMP01	CTRL	STD01	STD02	STD03	STD04	STD05	STD06	STD07	STD08	STD09	STD10	Well ID
	0.000	0.217	0.311	0.250	0.239	0.222	S0.223	0.155	0.116	0.109	0.058	0.008	Raw Data
B	0.112	0.217	0.311	0.250	0.239	0.222	S0.223	0.155	0.116	0.109	0.058	0.008	Transform
	1.000	1.000	0.001	0.050	0.100	0.200	0.500	1.000	2.000	3.000	5.000	10.000	Conc/Dil
	1.770	0.147	0.004	0.054	0.077	0.128	0.124	0.700	1.645	1.904	5.308	(13.909)	Calc Conc
	SMP01	CTRL	STD01	STD02	STD03	STD04	STD05	STD06	STD07	STD08	STD09	STD10	Well ID
	0.000	0.222	0.320	0.257	0.259	0.252	S0.242	0.210	0.142	0.098	0.051	0.004	Raw Data
C	0.113	0.222	0.320	0.257	0.259	0.252	S0.242	0.210	0.142	0.098	0.051	0.004	Transform
	1.000	1.000	0.001	0.050	0.100	0.200	0.500	1.000	2.000	3.000	5.000	10.000	Conc/Dil
	1.752	0.128	0.002	0.042	0.039	0.050	0.070	0.179	0.939	2.389	6.084	(15.013)	Calc Conc
	SMP01	CTRL	STD01	STD02	STD03	STD04	STD05	STD06	STD07	STD08	STD09	STD10	Well ID
	0.000	0.234	0.330	0.281	0.257	0.234	S0.225	0.194	0.141	0.068	0.049	0.010	Raw Data
D	0.122	0.234	0.330	0.281	0.257	0.234	S0.225	0.194	0.141	0.068	0.049	0.010	Transform
	1.000	1.000	0.001	0.050	0.100	0.200	0.500	1.000	2.000	3.000	5.000	10.000	Conc/Dil
	1.449	0.089	0.001	0.017	0.042	0.089	0.117	0.273	0.960	4.361	6.325	(13.388)	Calc Conc
	SMP01	CTRL	STD01	STD02	STD03	STD04	STD05	STD06	STD07	STD08	STD09	STD10	Well ID
	0.000	0.223	0.317	0.274	0.242	0.223	S0.231	0.158	0.147	0.106	0.044	0.006	Raw Data
E	0.115	0.223	0.317	0.274	0.242	0.223	S0.231	0.158	0.147	0.106	0.044	0.006	Transform
	1.000	1.000	0.001	0.050	0.100	0.200	0.500	1.000	2.000	3.000	5.000	10.000	Conc/Dil
	1.697	0.124	0.003	0.023	0.070	0.124	0.098	0.654	0.840	2.026	6.969	(14.451)	Calc Conc
	SMP01	CTRL	STD01	STD02	STD03	STD04	STD05	STD06	STD07	STD08	STD09	STD10	Well ID
	0.000	0.227	0.309	0.271	0.252	0.220	S0.200	0.172	0.137	0.102	0.046	0.004	Raw Data
F	0.116	0.227	0.309	0.271	0.252	0.220	S0.200	0.172	0.137	0.102	0.046	0.004	Transform
	1.000	1.000	0.001	0.050	0.100	0.200	0.500	1.000	2.000	3.000	5.000	10.000	Conc/Dil
	1.662	0.110	0.005	0.025	0.050	0.135	0.233	0.471	1.048	2.201	6.704	(15.013)	Calc Conc
	SMP01	CTRL	STD01	STD02	STD03	STD04	STD05	STD06	STD07	STD08	STD09	STD10	Well ID
	0.000	0.232	0.325	0.248	0.223	0.230	S0.225	0.178	0.131	0.089	0.043	0.005	Raw Data
G	0.119	0.232	0.325	0.248	0.223	0.230	S0.225	0.178	0.131	0.089	0.043	0.005	Transform
	1.000	1.000	0.001	0.050	0.100	0.200	0.500	1.000	2.000	3.000	5.000	10.000	Conc/Dil
	1.560	0.095	0.002	0.057	0.124	0.101	0.117	0.407	1.194	2.869	7.105	(14.729)	Calc Conc
	SMP01	CTRL	STD01	STD02	STD03	STD04	STD05	STD06	STD07	STD08	STD09	STD10	Well ID
	0.000	0.221	OUT	0.206	0.205	0.205	S0.187	0.150	0.107	0.074	0.032	0.006	Raw Data
H	0.114	0.221		0.206	0.205	0.205	S0.187	0.150	0.107	0.074	0.032	0.006	Transform
	1.000	1.000	0.001	0.050	0.100	0.200	0.500	1.000	2.000	3.000	5.000	10.000	Conc/Dil
	1.733	0.131		0.199	0.204	0.204	0.326	0.785	1.985	3.873	8.784	(14.451)	Calc Conc

04/23/99 12:59:46 PM

Page 1 of 1

Matrix Report for an absorbance assay.

MATRIX REPORT

Protocol ID: Sample Fluoro #2
Plate ID: Sample Fluoro #2

Plate Read Time: 11/18/99 4:13:53 PM
Temperature: n/a

	01	02	03	04	05	06	07	08	09	10	11	12	
	BLK	BLK	SMP04	SMP04	SMP08	SMP08	SMP12	SMP12	SMP16	SMP16	SMP20	SMP20	Well ID
	2354	2415	27589	28451	95214	98512	64125	64512	55125	54985	12452	12684	Raw (1)
A	3625	3685	59784	60025	98562	98364	65845	66845	72564	72568	98568	98645	Raw (2)
	-20	41	25215	26077	92840	96138	61751	62138	52751	52611	10078	10310	Blank (1)
	7	67	56166	56407	94944	94746	62227	63227	68946	68950	94950	95027	Blank (2)
	27.000	26.000	30951.0	30330.0	2104.00	-1392.00	476.000	1089.00	16195.0	16339.0	84872.0	84717.0	Plt Trans
	BLK	BLK	SMP04	SMP04	SMP08	SMP08	SMP12	SMP12	SMP16	SMP16	SMP20	SMP20	Well ID
	2301	2426	27985	27741	98564	97548	64356	64152	55157	54918	12485	12874	Raw (1)
B	3564	3598	58962	59236	99632	99759	66974	65145	73574	73048	98125	98265	Raw (2)
	-73	52	25611	25367	96190	95174	61982	61778	52783	52544	10111	10500	Blank (1)
	-54	-20	55344	55618	96014	96141	63356	61527	69956	69430	94507	94647	Blank (2)
	19.000	-72.000	29733.0	30251.0	-176.000	967.000	1374.00	-251.000	17173.0	16886.0	84396.0	84147.0	Plt Trans
	SMP01	SMP01	SMP05	SMP05	SMP09	SMP09	SMP13	SMP13	SMP17	SMP17	SMP21	SMP21	Well ID
	54785	51236	32174	32652	45784	45896	74125	74896	48741	48315	35482	35145	Raw (1)
C	95689	95326	47586	47965	78452	79045	57485	57564	53954	53784	29974	34574	Raw (2)
	52411	48862	29800	30278	43410	43522	71751	72522	46367	45941	33108	32771	Blank (1)
	92071	91708	43968	44347	74834	75427	53867	53946	50336	50166	26356	30956	Blank (2)
	39660.0	42846.0	14168.0	14069.0	31424.0	31905.0	-17884.0	-18576.0	3969.00	4225.00	-6752.00	-1815.00	Plt Trans
	SMP01	SMP01	SMP05	SMP05	SMP09	SMP09	SMP13	SMP13	SMP17	SMP17	SMP21	SMP21	Well ID
	53265	52412	32541	32895	45236	45512	73985	74035	48956	49258	35845	35987	Raw (1)
D	95748	95478	47512	46895	77985	78265	58024	58164	53165	54058	31545	30487	Raw (2)
	50891	50038	30167	30521	42862	43138	71611	71661	46582	46884	33471	33613	Blank (1)
	92130	91860	43894	43277	74367	74647	54406	54546	49547	50440	27927	26869	Blank (2)
	41239.0	41822.0	13727.0	12756.0	31505.0	31509.0	-17205.0	-17115.0	2965.00	3556.00	-5544.00	-6744.00	Plt Trans
	SMP02	SMP02	SMP06	SMP06	SMP10	SMP10	SMP14	SMP14	SMP18	SMP18	SMP22	SMP22	Well ID
	84125	84574	5265	5451	22658	22594	64256	64568	36315	36147	6859	6924	Raw (1)
E	65895	65623	64856	65874	46582	46832	44975	45065	66574	66256	48751	50524	Raw (2)
	81751	82200	2891	3077	20284	20220	61882	62194	33941	33773	4485	4550	Blank (1)
	62277	62005	61238	62256	42964	43214	41357	41447	62956	62638	45133	46906	Blank (2)
	-19474.0	-20195.0	58347.0	59179.0	22680.0	22994.0	-20525.0	-20747.0	29015.0	28865.0	40648.0	42356.0	Plt Trans
	SMP02	SMP02	SMP06	SMP06	SMP10	SMP10	SMP14	SMP14	SMP18	SMP18	SMP22	SMP22	Well ID
	85621	84997	5564	5326	22451	22365	63995	64015	35874	36284	6954	6895	Raw (1)
F	65712	65142	64789	64822	46854	46954	44541	44984	62457	64254	49624	49545	Raw (2)
	83247	82623	3190	2952	20077	19991	61621	61641	33500	33910	4580	4521	Blank (1)
	62094	61524	61171	61204	43236	43336	40923	41366	58839	60636	46006	45927	Blank (2)
	-21153.0	-21099.0	57981.0	58252.0	23159.0	23345.0	-20698.0	-20275.0	25339.0	26726.0	41426.0	41406.0	Plt Trans
	SMP03	SMP03	SMP07	SMP07	SMP11	SMP11	SMP15	SMP15	SMP19	SMP19	SMP23	SMP23	Well ID
	7512	7485	14785	14574	2945	2896	12235	12358	88247	87865	28456	28956	Raw (1)
G	44582	44236	35681	35225	62748	62954	37871	38452	60134	61455	84641	84345	Raw (2)
	5138	5111	12411	12200	571	522	9861	9984	85873	85491	26082	26582	Blank (1)
	40964	40618	32063	31607	59130	59336	34253	34834	56516	57837	81023	80727	Blank (2)
	35826.0	35507.0	19652.0	19407.0	58559.0	58814.0	24392.0	24850.0	-29357.0	-27654.0	54941.0	54145.0	Plt Trans
	SMP03	SMP03	SMP07	SMP07	SMP11	SMP11	SMP15	SMP15	SMP19	SMP19	SMP23	SMP23	Well ID
	7526	7495	14326	14621	2784	2985	12451	12953	88745	88315	28745	28415	Raw (1)
H	44689	44851	35488	35695	62478	62358	37849	37564	62345	61998	85624	86454	Raw (2)
	5152	5121	11952	12247	410	611	10077	10579	86371	85941	26371	26041	Blank (1)
	41071	41233	31870	32077	58860	58740	34231	33946	58727	58380	82006	82836	Blank (2)
	35919.0	36112.0	19918.0	19830.0	58450.0	58129.0	24154.0	23367.0	-27644.0	-27561.0	55635.0	56795.0	Plt Trans

11/18/99 4:29:46 PM

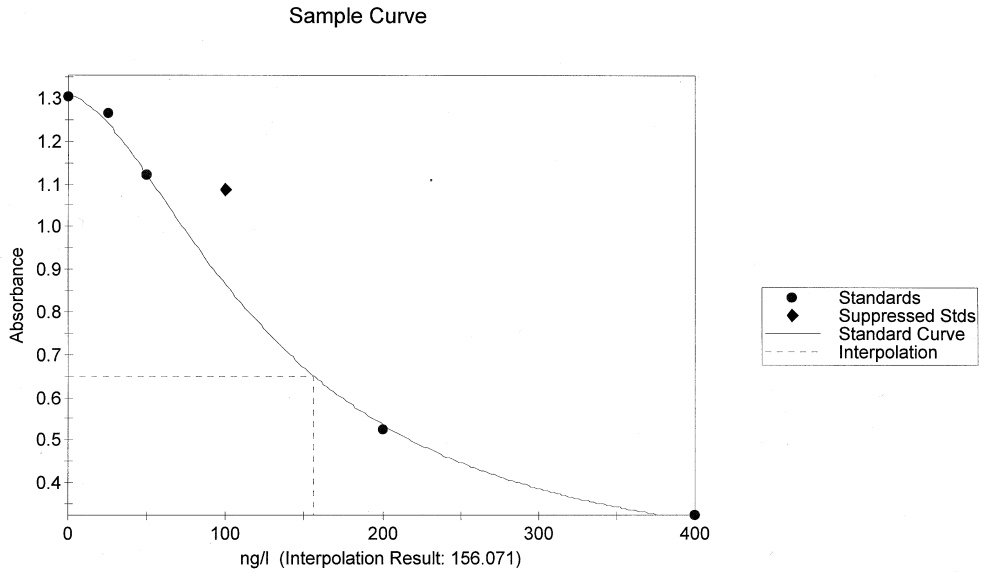
Page 1 of 1

Matrix Report for a fluorescence assay.

Sample Report
STANDARD CURVE REPORT

Protocol ID: Sample Protocol #7
Plate ID: Sample Plate #7

Plate Read Time: 06/23/98 08:56:16 AM
Temperature: n/a



2 Parameter (Logit-Log) (y = (A - D) / (1 + (x/C)^B) + D)
A=1.3050 B=1.7104 C=131.4776 D=0.1615, R-Square = 0.9987

04/23/99 01:17:04 PM

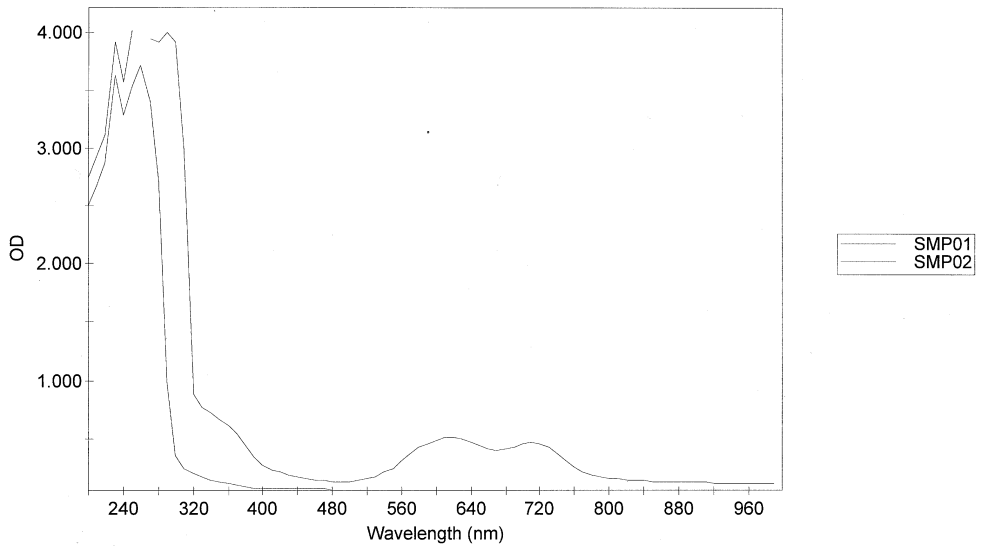
Page 1 of 1

Standard Curve Report.

SPECTRAL SCAN CURVE REPORT

Protocol ID: Sample Protocol #10
Plate ID: Sample Plate #10

Plate Read Time: 04/05/99 12:06:49 PM
Temperature: n/a



04/23/99 01:32:09 PM

Page 1 of 1

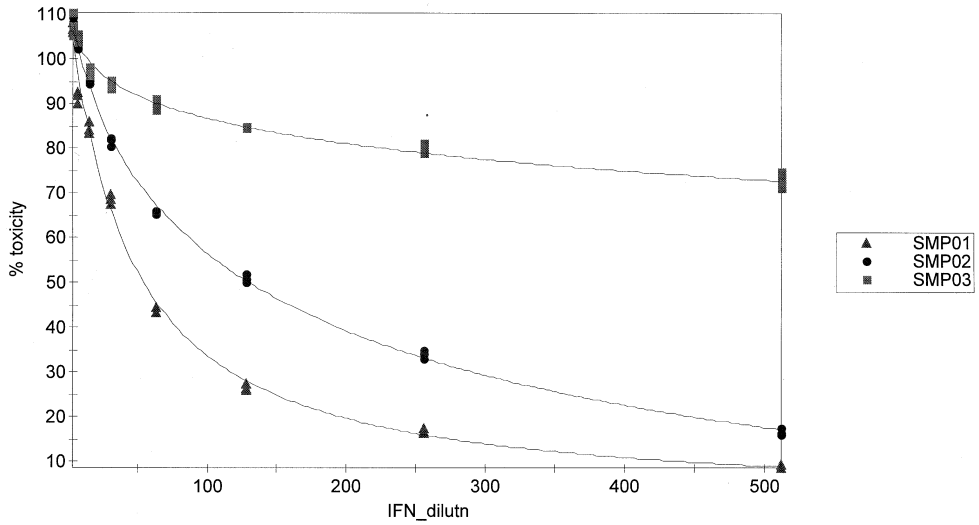
Curve Report showing two overlaid spectral scan curves.

Sample Report
TITER CURVE REPORT

Protocol ID: Sample Protocol #8
Plate ID: Sample Plate #8

Plate Read Time: 01/09/92 12:07:59 PM
Temperature: n/a

Sample Titer Curve Report



4 Parameter (y = (A - D) / (1 + (x/C)^B) + D)

04/23/99 01:22:31 PM

Page 1 of 1

Curve Report showing three overlaid titer curves.

Glossary

List of Terms

Abscissa

The coordinate representing the distance of a point from the y-Axis in a plane Cartesian coordinate system, measured along a line parallel to the x-Axis.

Ambiguous Curve

A curve is **ambiguous** if multiple concentrations exist for a given OD, RFU, or RLU value.

Average Standards

In a protocol, if standard groups are defined with multiple replicates, the **Average Standards** option is available.

- If **Average Standards** is *enabled*, KCjunior uses the average of the standard replicates when generating the curve.
- If **Average Standards** is *disabled*, KCjunior uses the individual standard replicates when generating the curve.

Coefficient of Variation (CV)

The **coefficient of variation** represents the standard deviation expressed as a percentage of the mean: (Standard Deviation / Mean) * 100.

In formulas, the coefficient of variation is represented by **CV( ).**

Database

A **database** is a computer file that contains multiple records of information.

In KCjunior:

- The **protocol database** contains multiple records called "protocols". Within each record (protocol) is a set of fields, containing information specific to that protocol, such as protocol description, read method type, and curve method type.
- The **results database** contains multiple records called "results records". Within each record is a set of fields, containing information specific to that plate, such as Results ID, Plate ID, read status, and raw plate data values.

Dilution

The amount by which a sample is mixed with a solution to bring the specie of interest into the operating range of the analysis.

Endpoint Read

During an endpoint **absorbance** read, every well on the plate is read with one (single) or two (dual) wavelengths.

- One optical density (OD) value is reported for each well.

During an endpoint **fluorescence** or **luminescence** read, the plate is read from one to six times, depending on the number of filter sets specified.

- One Relative Fluorescence Unit (RFU) or Relative Luminescence Unit (RLU) value is reported for each well for each filter set.

Extrapolation

KCjunior provides the option of displaying **extrapolated** concentrations – unknown values on the Y axis which are outside the range of the standards.

On data displays and reports, extrapolated concentrations are enclosed by parenthesis, for example **(20.345).**

Kinetic Read

During a kinetic **absorbance** read, every well on the plate is read a defined number of times with one (single) or two (dual) wavelengths.

- One optical density value is reported for each well for each plate read. In addition, a graph showing OD vs. Time is reported for each well.

During a kinetic **fluorescence** or **luminescence** read, the plate is read a defined number of times, using one filter set.

- One Relative Fluorescence Unit (RFU) or Relative Luminescence Unit (RLU) is reported for each well for each kinetic read. In addition, a graph showing RFU/RLU vs. Time is generated for each well.

MultiWavelength Read

During a **MultiWavelength read,** every well on the plate is read once at each defined wavelength (up to 6).

The optical density value measured at each wavelength is reported for each well.

Optical Density (OD)

The amount of light at a specific wavelength that is absorbed by the chromophore. It is the reciprocal of transmission (1/*T*).

Protocol

A **protocol** is one of many records in a protocol database containing user-defined information for performing a plate read and data reduction.

RFU/RLU

RFU stands for Relative Fluorescence Unit, **RLU** stands for Relative Luminescence Unit.

In KCjunior, if the current reader is set to a fluorescence/luminescence reader (such as the FLx800), the **Protocol|Read Method** dialog contains a **Chemistry** parameter.

If the Chemistry is set to **Fluorescence,** the plate measurement values are reported as RFU (ex. 87954 RFU). If the Chemistry is set to **Luminescence,** the plate measurement values are reported as RLU (ex. 57697 RLU).

R-Square

Also referred to as the Coefficient of Determination, **R-Square** indicates the strength of a regression function (curve fit). If the value is 1.0, then the fit is very strong, meaning that all of the points lie on the curve.

Standard Deviation (SD)

The **standard deviation** is a measure of how widely values are dispersed from the average value (the mean).

In formulas, standard deviation is represented by **SD( ).**

Template

In each KCjunior protocol, four unique **templates** can be designed to indicate location and related information for Well IDs, Dilutions, Transformations, and Sample IDs.

Transformations

Transformations are user-defined formulas applied to plate data for analysis, or for data transformation before calculating concentrations or cutoffs.

Two types of **transformations** can be defined within one protocol:

- A **transformation formula** is performed only on the well(s) to which it is assigned. If Transformation Formulas are applied, the results serve as the basis for curve generation and cutoff evaluation.
- A **plate formula** is automatically applied to every well on the plate that has been assigned a Well ID.

Well Identifier

KCjunior supports four different types of wells – Blank, Standard, Sample, and Control.

To accommodate a variety of applications, KCjunior provides the flexibility to customize references to these wells. These customized references are called **Well Identifiers.**

Index

2 Parameter curve 5:56
4 Parameter curve 5:56

A

Aborting a read in progress 6:9, 6:11, 10:9
Add to List button 5:48, 5:49
Addition (in a formula) 5:50
Adjusted value 7:37
Algorithm for auto sensitivity adjustment 7:33
Allow pathname selection at program start 4:12
Ambiguous curve 12:1
Apply to all wells 5:47, 5:48
Apply to selection 5:47, 5:48
Archive
  Archive protocol database 9:15
  Archive results database 9:15
  Archiving results records and protocols 9:15
  Archiving stored curves 9:17
  Archiving test results 9:18
  Creating archive databases 9:15
Auto sensitivity adjustment
  Algorithm 7:33
  Base value 5:30
  Defining the pre-reading parameters 5:28
  High value 5:30
  High well range 5:29
  Low well range 5:29
  Overview 5:28
  Test well range 5:29
  Use result for all filter sets 5:30
  Viewing results of pre-readings 7:32
Average standards 5:57, 12:1

B

Backup procedures 9:14
Bandwidth
  Defining in a filter set 5:25
  Warnings 5:25
Barcode scanning 4:10, 6:11
Basecode version 4:3
Base value 5:30
Baud rate 4:3, 4:4
Bio-Cell(tm) 5:17, 7:18
Bio-Tek, contacting 1:3
Blank Data 7:5, 7:9, 7:16
Blank limits, setting 5:9
Blanking method 5:39, 5:41
Blanks, defining 4:5, 5:38
Bottom optical probe 5:25

C

Calibrate before first read 5:37
Calibrating the reader 5:37, 6:8
Chemistry 5:21
Closing a results record 6:4
Coefficient of variation (CV) 5:50, 7:30, 12:1
Colors, changing 4:5, 4:9
Communication
  Connecting the reader to the computer 4:2
  Errors 6:17, 10:2
  Get communication defaults 4:4
  Port 4:4
  Reader system test 4:4, 9:8
  Testing communication 4:5
Comparison value (OD) 5:33
Computer
  Minimum system requirements 2:1, 2:2
Conc x dilution 5:51
Concentrations
  Calculated concentrations 7:20
  Defining expected 5:42
  Viewing results 7:20
Configuring setup parameters 4:5, 5:39
Configuring well identifiers 4:7
Connecting the reader to the computer 4:2
Constant value 5:18
Contacting Bio-Tek Instruments, Inc. 1:3
Continuous shake 5:35

Control panel, dispenser 9:4
Controls, defining 4:6, 5:38
Copying a results record 6:4
Copying formulas 5:47
Copying plate data to another application 6:24
Corrected absorbance 5:18
Create archive database 9:16
Cubic curve 5:56
Cubic spline curve 5:56
Current reader menu 3:6, 4:2
Current reader status 3:6
Curve
  Average Standards 5:57
  Curve Method 5:56
  Editing parameters 7:21
  Extrapolate 5:57, 7:2
  Graph Report Name 5:56, 7:21
  Outliers 7:25
  Overlaying 7:22
  Printing 5:63, 7:25
  Report 5:61
  Sample curves 7:22
  Spectral scan 7:11
  Standard 5:55, 7:21
  Stored 5:55
  Titer 5:55, 7:22
  X and Y Axes Label, Scale 5:57
Cutoffs
  Analyzing results 7:27
  Defining 5:58
  Labels 5:58, 7:27
CV function 5:50, 12:1

D

Data bits 4:3, 4:4
Data reduction 3:10, 7:1-7:29
  Errors 10:5, 10:7, 10:12
Database 4:9, 5:2, 5:5, 6:2, 6:5, 12:1
Database maintenance
  A note on backup procedures 9:14
  Archiving results records and protocols 9:15
  Archiving stored curves 9:17
  Archiving test results 9:18
  Creating archive databases 9:15
  Deleting results records and protocols 9:19
  Deleting stored curves 9:20
  Deleting stored transformations 9:21
  Deleting test results 9:22
Database version 4:10
Decimal places, changing the number of 4:8, 5:9
Delay before reading 5:32
Delay before sampling 5:28
Delay between samples 5:28
Delete after archive 6:5, 9:17, 9:18
Delimiter, choosing for data export 6:18
Delta OD 5:16, 6:12, 7:4, 7:5
DILution function 5:51
Dilutions
  Defined 12:1
  Defining 5:44
  Valid dilution range 5:45
Dispense / Read
  Read method type 5:14, 5:21
  Viewing results 7:14
Dispenser (FLx800)
  Control panel 9:4
  Filter position requirements 4:14
  Initializing 9:5
  Injector Position 9:7
  Priming 9:6
  Purging 9:6
  Syringe setup 9:7
Display curve legend 4:9
Display settings 2:2
Displayed decimal places, 5:9
Division (in a formula) 5:50
Dual-reader support 4:1, 4:2, 5:3

E

Editing standard outliers 7:25
Eject plate between filter sets 5:30
Emission filter 5:24, 5:25
Emission filter wheel 4:11, 5:23
EL301 microstrip reader 6:14
Empty plate, MultiWavelength read 5:16
End of transmission character 4:4
Endpoint read 5:10
  Defined 12:1
  Multiple endpoint reads 5:10, 5:20
  Results 7:4
EOT character 4:4
Error code, retrieving from reader 3:6
Errors and warnings
  A memory allocation error has occurred 10:7
  Ambiguous concentrations 10:7
  BTI shared library could not be found 10:7
  Calculation could not be performed 10:5
  Cutoff could not be calculated 10:7
  Cutoff is not greater than previous 10:8
  DIV/0 7:2, 10:8
  Error during auto sensitivity 10:8
  Extrapolated concentration 10:6
  Grayed numbers indicate a suppressed standard 10:9

Illegal log function attempted 10:9
Insufficient valid standards 10:9
No valid blanks in sector 10:9
OUT 10:10
Pathlengths could not be generated 10:10
Plate was aborted during read 10:10
Reader control error occurred during read 10:10
Reader error 10:2
Reader Status Failure 10:2
Reader Timeout Error 10:3
Serial Read Error 10:3
Serial Write Error 10:3
Stored curve does not exist 10:11
Strikethrough indicates masked well 10:11
Too many standards for curve type 10:11
Unable to generate curve 10:12
Unable to initialize curve standards 10:12
Well read error 10:7
Well was set to maximum valid value 10:5
Well was set to minimum valid value 10:6
Excel, exporting data to 6:21
Excitation filter 5:24, 5:25
Excitation filter wheel 4:11, 5:23
Extrapolate 5:57, 7:2
Exporting data
Copying results data and pasting it in another application 6:24
Delimiters 6:18
Directly to Microsoft Excel 6:21
Including an empty row or blank line 6:18
Including text headers 6:18
Specifying the first Excel cell location 6:18
To a text file 6:18
To an existing Excel spreadsheet 6:18

F

Fast read 6:7
Fastest kinetic interval 5:31
Filter position requirements for Dispense / Read protocols 4:14
Filter set (FS) function 5:51, 7:19, 7:20, 7:27, 7:29
Filter sets
Auto sensitivity 5:24, 5:28
Defining 5:24
Optics position 5:25
Options 5:27
Referencing in a formula 5:51
Sampling 5:27
Selecting the set for curve calculations 5:51, 5:56
Sensitivity 5:26
Filter wheel cartridges
Configuration 4:11
Emission 4:11, 5:23
Excitation 4:11, 5:23
Selecting at run-time 6:17
Selecting within a protocol 5:23
Filters, retrieving from reader 5:36
First Excel cell location for data export 6:18
Fluorescence
Auto sensitivity results 7:32
Blanked Data 7:16
Chemistry 5:21
Dispense / Read 5:10, 5:21
Endpoint read 5:10
Kinetic read 5:10
Raw Data 7:4
Relative Fluorescence Unit (RFU) 12:1
FLx800
Configuring filter wheel cartridges 4:11
Filter set definition in protocol 5:24
Filter wheel cartridge selection in protocol 5:23
FLx800 dispenser
Filter position requirements 4:14
Initializing 9:5
Injector position 9:7
Priming 9:6
Purging 9:6
Syringe setup 9:7
Formula
Coefficient of variation (CV) 5:50
Copying from well to well 5:47
DIL function 5:51
Help with creating 5:47, 5:49, 5:60
Filter sets (FS) 5:51
MEAN function 5:51
Order of precedence 5:53
Plate formula 5:49
Saving 5:48, 5:49
Standard deviation (SD) 5:52
Syntax rules 5:50
Transformation formula 5:47
Validating 5:48, 5:49
Value of current well (X) 5:53
Viewing results 7:19, 7:29
Wavelength (WV) 5:52
Well location reference [ ] 5:52
Well reference <well ID> 5:53
FS (filter set) function 5:51, 7:19, 7:20, 7:27, 7:29

G

Get basecode version 4:3
Get communication defaults 4:4
Get current reader status 3:6
Get reader wavelengths 5:16, 5:36

Getting Help 3:8
Glossary 12:1
Graph
  Copying 7:23, 7:25
  Moving, scaling, zooming 7:24
Printing 5:61, 7:23, 7:25
Graph interpolation result 4:8
Graphing options
  Axes scaling 7:24
  Borders and colors 7:24
  Legends and labels 7:24
  Spectral scan curve display options 7:12
Guided tour 2:15, 3:12

H

Help menu 3:7
Help system 3:8
High value 5:30
High well range 5:29

I

Importing
  Protocols 5:6
  Results records 6:5, 6:6
Incubation
  Defining read method parameters 5:34
  Incubation control utility 9:2
  Override button 9:3
  Setting a delay before reading 5:32
Inject on interval 5:23
Interpolation
  Calculate interpolation value 5:57
  Graph interpolation result 4:9
  Interpolation result 7:21, 7:22, 7:23

K

KCJr. for DOS plate files, opening 6:3, 6:13
Kinetic
  Fastest interval 5:31
  Interval 5:31
  Max slope unit of measure 7:8, 7:9
  Reads, number of 5:31
Kinetic read 5:10
  Defined 12:1
  Results 7:5

L

Labels 5:58, 7:27
Lag time 5:22
Legend, displaying in graph 4:9, 7:21, 7:22, 7:23
Lin or Log axes 5:56, 5:57, 7:24, 7:25
Linear curve 5:56
Logit Log curve 5:56
Low well range 5:29
Luminescence
  Auto sensitivity results 7:32
  Blanked Data 7:16
  Chemistry 5:21
  Dispense / Read 5:10, 5:21
  Endpoint read 5:10
  Kinetic read 5:10
  Raw Data 7:4
  Relative Luminescence Unit (RLU) 12:1

M

Masking reads, wells 7:37
Maximum slope
  Curves 3:11, 5:55
  Cutoffs 3:11, 5:58
  Statistics 7:30
  Transformations 3:11, 5:46
  Unit of measure 7:8, 7:9
MEAN function 5:51
Microsoft Excel, exporting plate data to 6:18
Microsoft Windows operating system 2:2
Minimum and maximum raw and blanked data values 5:8, 5:9
Monitoring wells
  Defining the criteria 5:33
  Elapsed time 7:35
  Selecting the well(s) to monitor 5:34
Multiple-plate endpoint read 5:20, 6:10
Multiple wavelengths
  Empty plate 5:16
  Pathlength correction 3:10, 5:17
  Referencing in a formula (WV) 5:52
  Selecting 5:16
  Selecting the set for curve calculation 5:52, 5:55
  Viewing results 7:4, 7:5
Multiplication (in a formula) 5:50
MultiWavelength read 5:10, 12:1

N

Network, sharing databases 5:5, 6:5
Number of plates 5:10, 5:20
Number of samples 5:27

O

OD 5:9, 12:1
OMNI System absorbance files, opening 6:3, 6:13
Opening a results record 6:2
Optical density 5:9, 12:1
Optics position 5:25
Optimum sensitivity 5:26, 5:28
OUT indication 6:15, 7:2, 7:37, 10:5
Outliers, editing 7:21, 7:25
Output empty row between plates 6:18
Output text headers 6:18
'Over' indication 7:37

P

Parenthesis, using in a formula 5:50
Parity 4:4
Partial plate reading 5:32
Password, obtaining from Bio-Tek 2:16, 2:17
Pathlength correction 3:10, 5:17, 7:17
Pathnames 4:5, 4:10, 4:12, 5:2, 5:6
Peak OD 7:11
Peak wavelength 9:12
Peak wavelength test 9:10
Plate
  Closing 6:4
  Copying 6:4
  Description 6:8, 6:10, 6:12, 6:13, 7:35
  Exporting data 6:19
  Geometry 5:32
  ID 4:10, 5:20, 7:35, 6:8, 6:9, 9:10
  Importing 6:5
  Opening a results record 6:2
  Partial plate reading 5:32
  Plate status 7:35
  Reading 6:15
  Saving 6:4
  Shaking 5:34
Plate formula 5:46, 5:49
Plate geometry 5:32
Plate ID 4:10, 5:20, 6:9, 6:11, 6:18
  Multi-plate read 6:9, 6:10, 6:11
Plate information 7:34
Plate mode reading 5:21
Point-to-Point curve 5:56
PowerWaveX reader models 2:14, 4:3
Primary wavelength 5:16
Printing reports 5:61, 8:1
  Print preview 8:1–8:2
  Print results 8:2
  Print setup 8:3
Product information 1:3
Protocol
  Blank limits 5:9
  Copying 5:5
  Defined 3:9
  Deleting 9:19
  Description 5:8
  Importing 5:6
  Name 5:8
  Raw data limits 5:8
  Read Method Type 3:11, 5:10
  Report Options 5:8
  Saving and closing 5:4
  Sharing protocols among users 5:5
Protocol database
  Archive protocol database 9:15
  Creating an empty database 5:5
  Importing protocols 5:6
  Pathnames 4:10
  PROTOCOL.MDB 4:10
  Sharing protocols and databases 5:5
  Version 4:12

Q

Quadratic curve 5:56

R

Rapid read mode 5:35
Raw Data 7:4
  Printing 8:1
  Viewing results 7:1
Raw data limits, setting 5:8
Raw Data report
  Selecting 5:61
  Printing 8:1
Read from file 6:3, 6:13
Read full plate 5:32
Read Method
  Auto sensitivity adjustment 5:28
  Calibrate before first read 5:37
  Calibrate Reader 5:37, 6:8
  Chemistry 5:21
  Delay Before Read 5:32
  Fastest kinetic interval 5:31
  Filter sets 5:25
  Get Reader Wavelengths 5:16, 5:36

Kinetic Interval 5:31
Kinetic Reads 5:31
Monitor well 5:32
Number of Plates 5:10, 5:20
Plate Geometry 5:31
Read Method Type 3:11, 5:10
Sampling 5:27
Sensitivity 5:26
Total kinetic read time 5:31
Validating parameters 5:31
Wavelengths 5:16
Read mode, selecting 5:35
Reader 1 or 2 4:1
Reader calibration 5:37, 6:8
Reader errors 7:35, 10:2, 10:10
Reader setup
Baud Rate 4:4
Com Port 4:4
Data Bits 4:4
EOT Character 4:4
Get Comm Defaults 4:4
Parity 4:4
Reader Type 2:14, 4:3, 5:10
Stop Bits 4:4
Test Communications 4:5
Reader system test 9:8
Reader system test history 9:9
Reader type 2:14, 4:3, 5:10
Reading a plate 6:13, 6:15
Reading a plate with a protocol 6:10
Reading a plate without a protocol 6:7
Reading plate data from a file 6:3, 6:13
Reconfiguring pathnames 4:5, 4:10, 5:2, 5:6
Reference wavelength 5:16
Referencing a well location in a formula 5:52
Registering KCjunior 2:15
Relative fluorescence/luminescence units 12:1
Removing KCjunior from your computer 2:3
Report options 5:8
Reports
Available types 5:61, 11:1
Display curve legend 4:9
Graph interpolation result 4:9
Print preview/setup 8:1–8:3
Print results 8:2
Printing 8:1
Sample printouts 11:1
Selecting in a protocol 5:61
Results
Analyzing 6:24
Auto sensitivity results 7:32
Blanked Data 7:16
Concentrations 7:20
Cutoff values 7:27
Database 4:9, 4:10, 6:2
Labels 7:27
Manipulating 7:34
Pathlength correction 7:17
Plate Formula Results 7:29
Printing reports 8:1
Raw Data 7:1, 7:4
Results records 6:2
Statistics 7:30
Template 7:28
Transformation Results 7:19
Viewing 6:24
Viewing statistics 7:30
Results database 2:10
Archive results database 9:14
Creating an empty database 6:5
Importing results 6:6
Pathnames 4:10
RESULTS.MDB 4:10
Sharing results records and databases 6:5
Version 4:12
Results ID 5:20, 6:2, 6:8, 6:9, 6:10
Results record
Copying 6:4
Deleting 9:19
Exporting data 6:18
ID 6:2, 6:8, 6:9, 6:10
Importing 6:5, 6:6
Opening 6:2
Saving 6:4, 6:9, 6:11
Sharing records and databases 6:5
RFU/RLU 12:1
R-square 12:1

S

Sample curves 7:22
Sample interval 5:21
Sample names, assigning 5:54
Sample protocols and results 2:15
Sample reports, 11:1
Samples
Defining 4:5, 5:38
Identifying 5:54
Sampling 5:27
Delay before sampling 5:28
Delay between samples 5:28
Number of samples 5:27
Sampling windows 5:22, 9:5
Saving a results record 6:4, 6:9, 6:11
Scanning a plate barcode label 4:10, 6:9, 6:11
Scientific notation 5:9, 5:10

Screen resolution 2:2
SD (standard deviation) function 5:52, 12:1
Select cartridges 5:23
Sensitivity
  Auto sensitivity adjustment 5:28
  Optimum sensitivity 5:26, 5:28
  Specifying within a filter set 5:26
Setup configuration 4:5, 5:39
Setup Menu 3:6
Shake
  Continuous during kinetics 5:35
  Defining parameters 5:34
  Intensity 5:34
Method 5:34
Sharing protocols and databases 5:5
Sharing results records and databases 6:5
Simulating a plate read 6:3, 6:12
Simulation data 6:3, 6:12, 6:24, 7:34, 7:38
Software registration 2:15
Software serial/version number 1:3
Spectral scan
  Adjusting the wavelength range used for calculations 7:12
  Calculation options 7:10
  Calibrate before first read 5:37
  Curve display options 7:12
  Peak OD 7:11
  Protocol definition 5:20
  Raw and blanked spectral data 7:9
  Spectral chart 7:10
Standard curve
  Editing 7:25
  Printing 5:61, 7:25
  Storing 7:26
  Titer curve 7:22
  Viewing 7:21
Standard deviation (SD) 5:52, 12:1
Standard outliers, editing 7:21, 7:25
Standards, defining 4:6, 5:38
Starting protocol 4:9
Statistics 7:30
Status
  Plate status values 7:35
  Read status values 7:37
Stop bits 4:4
Stopping a read 6:9, 6:11, 10:9
Stored curves
  Archiving 9:8
  Deleting from database 9:21
  Use stored curve 5:56
Storing a standard curve 7:26
Subtraction (in a formula) 5:50
Surge protector 4:2
Sweep mode 5:35
Symbols, in results 10:5
System configuration 4:5, 5:39
System requirements, minimum 2:2
System test, reader 9:8

T

Technical support 1:3
Temperature, setting 5:34, 9:1-9:2
Template
  Assigning well IDs 5:38
  Dilutions 5:44
  Sample Ids 5:54
  Transformations 5:46
  Viewing results 7:28
Test well range 5:29
Testing communication 4:5
Tip prime option 5:22
Titer
  Curves 7:22, 7:26
  Defining dilutions 5:44
  Interpolation value 5:57
  Statistics 7:30
Top optical probe 5:25
Total kinetic read time 5:31
Transformations
  Defining 5:46
  Deleting stored 9:22
  Formula syntax 5:50
  Plate formula 5:46, 5:49
  Transformation formula 5:46, 5:47
Tutorial 2:15, 3:12

U

'Under' indication 7:37
Uninstalling KCjunior 2:3
Universal plate test 9:10
Universal plate test history 9:14
Use result for all filter sets 5:30
Utilities
  Carrier Out/In 9:4
  Database maintenance 9:14
  Diagnostics 9:8–9:13
  Dispenser control panel 9:4-9:7
  Incubation control 9:1–9:2
  Reader system test 9:8
  Universal plate test 9:10
Utilities menu 3:5

V

Validate a formula 5:48, 5:49
Validate read method parameters 5:31
View menu 3:7

W

Wavelength function (WV) 5:52, 7:19, 7:20, 7:27, 7:29
Wavelengths
  Multiple 5:16
  Primary 5:16
  Reference 5:16
  Retrieving from reader 5:16, 5:36
Well identifiers 4:5, 5:38
Well IDs
  Assigning 5:38, 5:39
  Defining defaults 5:39
  Modifying an existing template 5:43
  Referencing in a formula 5:53
  Template 5:38
Well information 7:36
Well location, referencing in a formula 5:52
Well mode reading 5:21
Well notations 7:1, 7:2
Well reference [ ] 5:52
Well type colors 4:5, 4:9
Windows operating system 2:2
(WV) wavelength function 5:52, 7:19, 7:20, 7:27, 7:29

X

X (in a formula) 5:53